VIII. *The Process of Secretion in the Skin of the Common Eel.*

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Communicated by Professor M. Foster, *Sec. R.S.*

Received April 18,—Read June 8, 1893.

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Introduction.

We owe to Leydig (1) the first demonstration that a secretory process is a possibility for the skin of the Fish. In some twelve genera he described the occurrence of specialized cells in the epidermis, to which he gave the common name of "Schleimzellen," though subsequent research has shown that several varieties of structure were included in this generic term.

Kölliker (2) in 1860, in an investigation of the skins of *Myxine* and *Petromyzon*, differentiated two cell-forms. Observing the "thread bodies" of the mucous sacs of *Myxine*, first clearly described by Johannes Müller (3), he recognized their cellular nature, and found that the epidermis held similar, though less complex cells, from whose contents also, by appropriate treatment, a thread could be obtained. To these epidermic cells of *Myxine* the name "Fadenzellen" was applied. In addition, clear or finely granular cells, termed "Schleimzellen," were described.

The cells in the epidermis of *Petromyzon*, considered as the analogues of the "Schleimzellen" of *Myxine*, were described as "keulenförmige," with a vesicular space at the swollen end holding a pair of nuclei, and with elongated neck, "mit zarten, häufig leicht wellenförmig gebogenen Längstreifen versehen." A second special form was taken as corresponding with the "Fadenzell." Globular in shape, but supplied with a long thread-like process, it appeared to be filled with granules, which, however, were considered by Kölliker to be the optical expression of a closely-wound thread included in the cell. The name "Körnerzellen" was given to these structures. A secretory function was considered probable for the "Schleimzellen" of both *Myxine* and *Petromyzon*, but there are no direct observations upon the specific point.

A year later, Max Schultze (4) reinvestigated the structure of the skin of the same two Fish.

In the case of *Petromyzon* he renamed Kölliker's "Schleimzellen," "Kolbenformige Gebilde" or "Kolben," emphasized the occurrence of longitudinal striation in these structures, but also added a transverse striation of the neck, insisted upon as not superficial, but produced by alternating discs of isotropous and anisotropous material—an appearance, however, absent in the fresh specimen. These "Kolben" were described as always in contact with the corium by their lower ends, and at this point a connection with a nerve filament was considered as possible. Indeed, Max Schultze looked upon these structures as of the nature of nervous end-organs, possibly

contractile, on account of the points of similarity to striated muscle fibre presented by the neck viewed by polarized light.

Similar structures were also briefly described for the epidermis of the Eel and Tench.

MAX SCHULTZE further showed that these "Kolben" are the analogues of the "Fadenzellen" of *Myxine*, and not of the "Schleimzellen," as KÖLLIKER supposed, the "Körnerzellen" being identical with the latter.

HEINRICH MÜLLER (5), in 1864, was the first to observe in *Petromyzon planeri* that the "club cells" (Kolben) do not necessarily retain their connection with the corium, as MAX SCHULTZE stated. From observations of the variations in number, shape, and position in the epidermis, he supposed that these cells are liable to extrusion. Thus (p. 44): "Es findet diess in der Art statt, dass es sehr nahe liegt, eine *Abstossung der Gebilde mit oder ohne Wiedersatz* anzunehmen" (*italics* in original). He did not, however, actually observe this extrusion. He expresses himself as opposed to the assumption of a nervous connection, and considers these structures as modified epithelial cells of uncertain function.

The work of KÖLLIKER, MAX SCHULTZE, and H. MÜLLER had been chiefly upon the three species of *Petromyzon*, and, in the case of the two former observers, upon *Myxine* also.

F. E. SCHULZE (6), in 1867, extended our knowledge of the epidermis of Fish by an investigation of fifteen genera, including *Petromyzon*. Three forms of specialized cells—one of which, however, was found peculiar to *Petromyzon*—were described.

(1.) "Becherzellen," found in all the genera taken, characterised by possession of a distinct "theca," filled in the fresh state with granules imbedded in a more fluid mass, and whose secretory activity was actually observed and figured in the case of the barbel of *Cobitis fossilis* (Plate 7, fig. 3), and seen also in the skin of the caudal fin and head of small Eels. An account is, moreover, given of an Eel dropped upon a sandy floor, relieving itself of the casing of adherent grit, in the form of a tube with slimy interior, after replacement in water.

(2.) "Kolben," seen only in *Tinca*, *Leuciscus*, *Cobitis*, *Anguilla*, *Esox*, *Silurus*, and *Petromyzon*.

(LEYDIG (1) had previously investigated the first five of these genera, and, since he gave no special description of "club cells," must have included both "Kolben" and "Becherzellen" under the one term "Schleimzellen.")

In dealing with the important point as to whether the "club cells" are permanently fixed to the cutis or capable of passage to the surface, F. E. SCHULZE describes them as breaking away from the cutis in all cases except in the Lamprey and Eel. (He figures, however, a case in the Eel in Plate 7, fig. 7, but considers it pathological.) In the Tench the "club cells" near the surface become irregularly rounded or flattened, and the possibility of their extrusion is mentioned. Thus, p. 160: "Solche veränderte Kolben habe ich vielfach dicht unter der äussersten Zellenlage gefunden, so dass wohl

kein Zweifel darüber bestehen kann, dass sie beim Ausfallen einer darüberliegenden Zelle selbst auf die Oberfläche des Fisches gelangen."

F. E. SCHULZE considered the "club cells" as secretory in nature, and as possibly forming a sebaceous matter (Talgdrüsen). He could not substantiate MAX SCHULTZE'S statement regarding the presence of transverse striation in the neck.

(3.) The "Körnerzellen" of the Lamprey were more closely described by F. E. SCHULZE, their processes being considered as due to tubular prolongations of a cell membrane, yet as passing within the body of the cell to become united by a junction piece resembling the head of a pair of compasses. They were considered to be sense cells, but no nerve connection was demonstrated.

LANGERHANS (7), in his monograph on *Petromyzon planeri*, failed to observe the "club cells" clear of the corium. The most interesting point, however, in his description of the skin of this Fish, is the recognition of a form of cell not hitherto described. Under the name "kleine Rundzellen," pp. 16 and 17, he notes cells resembling lymphocytes as occurring between the other elements of the epidermis. These are figured in Plate 1, fig. 11, as of from 4 μ to 5 μ in diameter, and possessed of scanty protoplasm. He considers that these cells represent chromatophores which have not developed pigment.

To A. FŒTTINGER (8) (1876) is due a valuable paper upon the epidermis of *Petromyzon*. The most important point in connection with this paper is the complete corroboration of HEINRICH MÜLLER'S statement as regards solution of continuity between the "club cells" and the corium, and, further, the actual demonstration that these bodies escape from the epidermis, and may be found lying upon its surface (Plate 2, figs. 8, 9, 10, 11, 12; Plate 3, fig. 37). Thus, p. 629: "la massue entière ou tout au moins la partie qui se colore en jaune par le picrocarminate d'ammoniaque est eliminée." The process of extrusion was carefully followed.

A vacuolation between the surface of the club and an enveloping membrane is said to occur, loosening it by the formation of a network of granular material (Plate 2, fig. 1). An accumulation of fluid in the spaces of this network, especially at its lower part, is considered to force the "club cell" upwards, leaving its membrane *in situ*. In the passage out the nuclei disappear, and are always absent from the extruded cell.

He agrees with F. E. SCHULZE as regards the structure of the goblet cells, but does not agree with this observer as regards the nervous nature of the "Körnerzellen."

The characters of the goblet cells common to the epidermis of all Fish have been especially described by LIST (9), who, while describing the "footed" and "footless" forms of F. E. SCHULZE, further subdivides the latter into "stalked" and "unstalked" varieties, the stalk being merely a prolongation of the theca of the cell.

LIST differentiates the contents of the theca into a "filar mass" arranged as a net and staining easily, and a homogeneous "interfilar mass" in the meshes of the former. He considers that the contents of the theca are extruded, when the goblet

reaches the surface by a turgor of the interfilar material as the result of imbibition of water.

LEYDIG (10) in a later paper has given a few more details of interest in connection with this communication.

In the lower layers of the epidermis of two Batrachian larvæ (*Pelobates fuscus* and *Hyla arborea*), he describes and figures cells holding much convoluted threads, which occasionally pass beyond the border of the cell. He compares these to the "Fadenzellen" of *Myxine*, and terms them "Byssuszellen." EBERTH (11) had previously described very similar cells in the epidermis of the larva of *Bombinator igneus* (see especially Plate 25, figs. 19 and 24, of his paper).

LEYDIG admits the membrane described by FŒTTINGER for the "club cells," and ascribes MAX SCHULTZE'S transverse striation to folds of this structure (Plate 7, fig. 7). He notes, too, in the "club cells" of *Petromyzon marinus* an appearance of tangled threads. He considers both the "club cells" and "Körnerzellen" of *Petromyzon* as secretory in nature.

FRITSCH (12), in his account of the epidermis of *Malapterurus*, describes four varieties of cells: (*a*) "Kolbenzellen," (*b*) "Becherzellen," (*c*) "gewöhnliche Epidermiszellen," and (*d*) "Kornzellen."

He considers the "club cells" to be secretory in function, and in his figure (Plate 8, fig. 25) indicates a point where these cells have escaped from the epidermis, and also "club cells," the contents of whose "vacuoles" have been discharged.

The goblet cells arise, he thinks, from the ordinary epidermic cells of the middle layers. The ordinary epidermic cells are characterised by the possession of a "vesicular" nucleus which is poor in chromatin, and in strong contrast to that of the fourth variety, viz., the "Kornzellen." These "Kornzellen," which are evidently the "kleine Rundzellen" of LANGERHANS (7), are very similar to ordinary lymphocytes. LIST (13) has figured similar cells in the epidermis of *Cobitis fossilis*, and considers that they are of the nature of the wandering cells described by STOHR (14) in the surface epithelium of the tonsil.

FRITSCH makes no very definite statement of opinion as regards the origin and fate of these cells. In his description of Plate 8, fig. 25, he marks them *Leucocyten?* yet in the text he expresses himself as opposed to LIST'S hypothesis that they are wandering cells in the ordinary sense of the term, since he failed, in contradistinction to the latter observer, to find these cells between the fibres of the corium.

FRITSCH did not see any evidences of division of these cells, though LIST figures forms which he takes to represent such a process.

On the whole, he inclines to the rather curious view that they supply the most superficial epidermic scales.

Still more recently POGOJEFF (15), who appears to have been ignorant of the work of FŒTTINGER, has written on the structure of the epidermis of *Petromyzon*. By the use of gold chloride he sees a violet staining fibril within each "club cell" connected

with two cells, the whole being surrounded by endothelial sheaths after the manner of a Pacinian corpuscle. He thus agrees with the old idea of MAX SCHULTZE, that the "club cells" represent the end apparatus of cutaneous nerves, and considers that his failure to observe solution of continuity between the lower ends of the "club cells" and the corium supports this view. He strangely enough maintains that the statement that the "club cells" leave the corium has arisen from observation of oblique sections! He agrees with KÖLLIKER in considering the "Körnerzellen" of the epidermis of this Fish to be of the nature of unicellular glands.

It will be evident from the above brief account of the work of previous observers that an anatomical basis for a secretory process of considerable complexity and peculiar nature exists in the epidermis of many Fish. The skin, apart from its protective and sensory functions, which are not here under consideration, may also fairly be considered as a glandular surface of special construction, and the absence of localised collections of secretory cells provided with ducts such as we find in higher Vertebrates is no basis for the statement occasionally met with that "the skins of Fishes are non-glandular." Unicellular secreting structures whose function it is to supply the slime bedewing the surface of the animal are abundant in the epidermis, and in the special case of the Myxinoid Fishes these unicellular structures actually become collected to form glands in the commonly accepted sense of the term.

In none of the above researches is any mention made of the payment of special attention to the condition of secretory activity of the skin at the moment that the specimen was fixed for histological purpose. To this is to be attributed to a large extent the divergence of opinion concerning the functions of the "club cells" in particular. Nor, except in the well-known case of *Myxine* (32), has the fresh slime of the Fish been subjected to the microscope.

Chance stimulation, due to handling in capture, varying in amount in different cases, must have accelerated the normal secretory process more in some cases than in others, and produced a change in the histological picture and the physiological deduction therefrom. In no case, as far as I am aware, has direct experimental excitation been employed.

I propose in the following pages to give an account of the secretory process in the skin of the common Eel, deduced, in the first place, from the histological appearances at various levels of the epidermis and the formed elements of the slime, and, in the second place, from those changes that can be artificially produced by experimental irritation.

Special care has been bestowed upon the Fish in order that the condition of the skin at the time of preservation might be known.

The animals were always caught without a hook, and were kept some days after caturre in running water and supplied with food.

To obtain a skin in the *lowest phase of secretory action* hybernating sluggish Fish were employed, and death was effected by transfixion of the medulla. If this is skilfully performed the animal instantly becomes motionless, and pieces of skin may

be removed without reflex movement (and concomitant reflex secretory action) during the period of persistence of the condition of "shock."

A Fish that struggles, from failure to strike the right place at once, becomes a "sliming" Fish, and is useless for a picture of the slow process of normal secretion, though useful for that of some of the higher phases of the act.

The methods of artificial stimulation will be found under their appropriate headings, and the histological procedure in the Appendix.

§ 1. Formed Elements of the Slime of the Eel.

Seeing that the secretory structures of the skins of Fish are not provided with ducts, there is no method of escape for the secreted material, except by actual casting off of the superficial epidermic scales. The slime, therefore, always contains numbers of epidermic scales, unavoidably thrown off in the process of its formation.

For purpose of collection of the secreted matters, three methods have been employed—(1) Placing the animal in a bath of pilocarpine solution; (2) Subjecting the animal to faradization; (3) exposing the Eel to the vapour of chloroform. By the first and second methods it is possible to collect little "flocks" of secreted matter as they rise from the surface of the Fish; in the third method, the secretory activity is very energetic, and the passage of a blunt scalpel gently over the surface of the Fish removes abundant material.

Apart from the epidermic scales above mentioned, the following formed elements are present in the secretion collected by any of the above methods: (*a*) *Threads;* (*b*) *Nuclei;* (*c*) *Granules;* (*d*) *Goblet cells.*

(*a.*) *Threads of the Slime.*—These structures occur singly in much convoluted masses (Plate 31, fig. 14), or more extended, but generally in the form of masses of more finely fibrillated material (Plate 30, fig. 1). The extraordinarily tenacious character of the slime is undoubtedly associated with the presence of these threads.

The most pronounced case of a thread secretion in Vertebrates is, of course, the well-known instance of the Myxinoid Fishes, where the threads are known to arise by a metamorphosis of the specialized "Kolben," contained in the ventral slime glands (Kölliker (2), Max Schultze (4), also Blomfield (16)).

Thread-like structures within epidermic cells are also described, by Eberth (11), in the lower layer of the epidermis of the larva of *Bombinator igneus,* and, by Leydig (10), in the epidermis of the larva of *Pelobates fuscus* and *Hyla arborea,* though neither observer noted their escape in a secretion. An able account of many ectodermic thread secretions in Invertebrates and their possible relation to those found in Vertebrates has been given by Eisig (17), from which it is evident that such threads may be used for the construction of protective tubes (*Cereanthus,* certain Annelids, Insect larvæ), for catching prey (*Holothuria nigra,* Spiders), or for attachment to a fixed object (Byssus of Lamellibranchiate Molluscs). The thread of the

Sea Stickleback (*Gasterosteus spinachia*), used for nest building, has been shown, by MÖBIUS (18), to be a secretion of the renal epithelium.

The use of the threads in the secretion of the Eel appears to be to ensure that the slime shall adhere to any object with which it is brought in contact.

The threads have the following characters. The breadth of the coarsest may be from 2 μ to 3 μ, but all conditions of finer fibrillation are found, the finest fibrils being beyond accurate measurement.

Usually present in wavy masses of the finest fibrils entangling other elements, one often, especially when the pilocarpine method is used, comes across convoluted masses of the coarser variety. The extremities are often wound in a corkscrew fashion (Plate 30, fig. 1, on the right).

They are unchanged by induction shocks applied to fresh material. Do not swell in water. Readily give the xantho-proteic reaction. Resist digestion with pepsin and hydrochloric acid, and with trypsin and sodic carbonate. One per cent. sodic hydrate leaves them unaffected. Osmic acid darkens them slightly. Treated with picro-carmine, they stain brilliant yellow (Plate 30, fig. 1).

They are thus composed of some albuminoïd material of very resistant nature, possibly allied to keratin.

(*b.*) *Nuclei of the Slime.*—Free nuclei are frequent in the fresh slime; they vary in diameter from 2 μ to 4·5 μ, and are generally spherical, but occasionally ovoid (Plate 30, fig. 1).

No distinct chromatin network or filament was seen, though these nuclei take most dyes easily. In no case could a cell body be distinguished, though, at times, a group was met with surrounded by a flocculent material.

(*c.*) *Granules of the Slime.*—Small spherical granules (·75 μ to ·5 μ) abound in the fresh slime, and can be preserved upon cover-glass preparations. They present the following characters.

They give the xantho-proteic reaction. Are soluble in five per cent. acetic acid. Swell in one per cent. sodic hydrate, but do not dissolve. Do not dissolve easily in water (still clearly outlined in slime kept a day in water). Are not soluble in five per cent. sodic chloride solution containing five per cent. of acetic acid. Resist peptic and tryptic digestion. Are present on cover-glass preparations, after treatment with boiling alcohol and ether. In cover-glass preparations treated with corrosive sublimate they do not give the reddish-violet colour with thionin, considered, by HOYER (19), to be characteristic of mucin. They take the methyl green of the Biondi stain, prepared according to HEIDENHAIN'S (20) recipe. They also stain easily with soluble blue and carmine.*

* January, 1894. Since writing the above I have found that these granules stain deep brown when treated on a cover-glass with nitro-molybdic solution, followed by pyrogallol, according to the method of LILIENFELD and MONTI (33) for micro-chemical detection of phosphorus. They are possibly, therefore, of the nature of nucleo-albumin.

(*d.*) *Goblet cells.*—Extruded goblet cells occur in the slime, though rather rarely. Their characteristics will be entered into below in connection with the elements of the epidermis itself.

The epidermic scales found in the slime are flattened scales, devoid of "prickles"; the cell body, punctated with fine dark granules, measures from 15 $\mu \times 10\ \mu$ up to 23 $\mu \times 12\ \mu$; the nucleus is large and vesicular, and measures from 5 $\mu \times 4\ \mu$ to 12 $\mu \times 6\ \mu$. There is no evidence of any mucinous change in the protoplasm of these cells, and the cell bodies refuse to give the reddish-violet reaction with thionin.

All the above elements of the slime are seen in Plate 30, fig. 1.

In addition, however, mucin is undoubtedly present in "solution" in the slime, for dilute alkaline extracts give a precipitate with acetic acid, insoluble in excess, and upon long boiling with two per cent. H_2SO_4, a substance reducing FEHLING'S fluid is formed. Furthermore, in cover-glass preparations, one gets streaks and nets of a material distinguishable from the threads by taking the carmine instead of the picric acid of picro-carmine, staining reddish-violet with thionin, and also giving the orange-red colour with saffranine, described, by PANETH (21), as characteristic of mucin.

The slime is alkaline to litmus, but, curiously, will not affect phenolphthalein, a point which I have also noticed with *Myxine* (37).

§ 2. THE STRUCTURE OF THE EPIDERMIS.

Before attempting to account for the origin of the elements of the slime just described, it is necessary to detail the structure of the epidermis. In this account I shall describe the epidermis as it appears in its lowest phase of secretory activity as prepared by the method mentioned on pp. 324 and 325, and this condition will be spoken of as "normal" in contrast to the condition evoked by artificial excitation, which will be designated "stimulated."

The structure of the normal epidermis has been investigated by the two methods of teasing and section-cutting, and will be described under two appropriate headings.

(α.) *Teased Preparations.*

Fresh teased preparations are not successful, the tenacious slimy nature of the epidermis not allowing sufficient separation of the elements. I have therefore resorted to maceration in RANVIER'S "third part" alcohol, with subsequent staining, and teasing in dilute glycerine, or making cover-glass preparations of the macerated material with subsequent staining.

(1.) *The club cells.*—These curious cells are always the most conspicuous element in teased preparations. The great variety of form and size of these bodies, indicating that they are subject to continual metamorphosis in the process of their development, will be evident from a glance at some of the forms shown in Plate 31.

A medium stage in development will be the most convenient point from which to commence description.

In such condition the club cells are pyriform with much elongated stalk, the total cell being from 50 μ to 100 μ in length, the greatest breadth 15 μ to 30 μ, tapering below to a blunt end varying from 2 μ to 10 μ across. The stalk is beset with small projections, giving it a "shaggy" appearance under the high power.

Within the upper expanded part of the club is seen a vesicle, bordered by a lattice work of clear material in stout struts, and filled with finely granular matter. This vesicle is as a rule ovoid, its longer axis coinciding with that of the club and measuring from 12 μ to 13 μ in the long, and 9 μ to 20 μ in the short diameter. One nucleus with distinct nucleolus is always present, resting against the wall of the vesicle, but two such nuclei often occur (figs. 15*a* and *b*, Plate 31). At the lower end of the vesicle the protoplasm of the stalk of the club presents a central core of material more granular than the peripheral parts, and this core may extend for variable distances into the stalk, often reaching nearly to the lower end.

It is not however necessary to find one vesicle only, two such may be present (figs. 16*a* and 16*b*, Plate 31), and in such cases each holds a nucleus.

As regards action of dyes, the most marked reaction is that with picro-carmine, in which the material of the stalk and walls of the vesicle stains brilliant yellow (similar to the threads of the slime), while the nucleus stains red, the contents of the vesicle usually remaining colourless. This reaction has already been noted by FŒTTINGER (8) for the club cells of *Petromyzon*. With the Biondi mixture, the body of the club takes the acid fuchsin, the contents of the vesicle and the nucleus the methyl green.

Among these comparatively simple club cells are found others which evidently represent further stages in development.

Apparently two somewhat different processes may occur, though in the end both lead to the same nett result, viz., the production from the cell body of a convoluted or spiral fibre ("fibre-mass") which is capable of subsequent further division into fibrils, and the extrusion of the contents of the vesicle in the process of development of the fibre.

In both processes the final result is obtained by the vacuolation and canalization of the material of the upper part of the body of the club, proceeding generally from below upwards and from the regions in the immediate proximity of the periphery of the vesicle towards the surface.

The variation in the process above alluded to, lies in this, that an incomplete shell of the original club body material may be left adherent round the vesicle, so that when set free it appears as a cell with nucleus and cell wall composed of the club cell body material (formation of "escape cell"), or, on the other hand, the vacuolation may be more complete and so free the contents of the vesicle with little adherent matter (formation of "escape mass").

The first process, viz., the formation of an "escape cell," is illustrated in figs. 17*a*, *b*,

and *c*, Plate 31. In 17*b* it is evident that the superficial lattice work round the original vesicle comes away with it as an outer coat to the "escape cell." It would appear probable that this lattice work is the result of the erosion of the club body material immediately in contact with the wall of the vesicle, in the process of vacuolation that leads up to its escape.

Cells bearing such a superficial lattice-work-like thickening of the cell membrane have been described in the epidermis of certain larval Amphibians, and are generally known as "LEYDIG'S cells." Originally described by LEYDIG (22) as "Schleimzellen" in the larva of *Proteus* and *Salamander*, the description in the latter animal was subsequently supplemented by LANGERHANS (23), FLEMMING (24), PFITZNER (25), and LIST (9). LIST described them also in the larva of *Triton*, and CARRIÈRE (26), and PAULICKI (27) in *Siredon pisciformis*, the latter observer naming them "Netzzellen." LEYDIG (28) too has more recently again given a description for the cells in larval *Salamander* (see especially his fig. 25, Plate 31).

The rib-like thickenings of the cell membrane were seen by PFITZNER in the living epidermis, and are not, therefore, due to a precipitation by reagents as FLEMMING supposed. FLEMMING and PAULICKI, moreover, showed that the nucleus of these cells is not necessarily "incised" as LANGERHANS originally described. The fate and function of the cells in larval Amphibians is quite unknown. They at any rate, however, belong exclusively to the period of aquatic life (PFITZNER and PAULICKI), and during that period undergo division (FLEMMING, PFITZNER, and PAULICKI).

These "escape cells" are frequently found isolated in teased preparations of the Eel's skin, and in many cases bear a striking resemblance to the figures of the LEYDIG'S cells of larval Amphibians given by the above authors.

Figs. 21, 22, and 23, Plate 31, are illustrative of the structure of these cells, and are all drawn to the same scale. The size is very variable, $65\,\mu \times 50\,\mu$ to $20\,\mu \times 12\,\mu$, the majority, however, having an average size of $40\,\mu \times 30\,\mu$. The lattice work takes the picric acid of picro-carmine, suggesting that it is of the same composition as the club cell body material, while the granular contents stain faintly pink. The larger specimens (figs. 21*a* and *b*) generally bear attached a convoluted piece of the "thread mass," to be shortly described. Occasionally these cells bear distinct traces of the method by which they have been shelled out by a process of vacuolation, bearing attached some of the bars of the lattice originally formed in the substance of the head of the club (figs. 22*a*, *b*, and *c*). Two nuclei are often present (figs. 22*b*, 23*a*), though whether this is due to origin from the vesicle of a bi-nuclear club or to subsequent division is not clear, though nuclei with two nucleoli (fig. 23*b*) would seem to suggest the latter as a possibility. I have never seen any mitotic figures, however, in these nuclei.

In other cases the nucleus is found on the point of being extruded (figs. 23*b*, *c*, and *d*).

In the second process of development of the club cell, viz., the production of an

"escape mass," the vacuolation of the material of the club head appears to be more extensive, and, instead of a special capsule remaining round the original vesicle, the whole is broken up, leading to setting free of the granular mass within. Such granular masses ("escape masses"), often still holding the original nucleus of the vesicle, are frequent in the teased preparations, and are evidently only a modification of the escape cells above described.

The process of vacuolation of the club head is illustrated in figs. 20*a*, *b*, and *c*, Plate 31, and it is at once evident from a glance at these how the "fibre mass," consisting of the remains of the club body material is formed by being "gutted out" of the head of the club, the stalk remaining attached.

A splendid example of an isolated "fibre mass" is figured in fig. 20*d*, Plate 31. It is to be noted that these fibre masses are always coiled or spirally wound (figs. 18*a* and *b*, 19*a* and *b*, 20*c* and *d*), suggestive of an "elater" action in removal of the surface epidermic scales to allow of escape of secretion; I have not, however, been able to convince myself of any hygroscopic property.

The "thread mass" thus formed is capable of further disintegration. Secondary vacuolation is often seen within it at its upper part (fig. 19*a*, Plate 31), and it is finally possible for it to be split into the finest fibrils similar to those found in the secretion (fig. 4*b*, Plate 30). These finely fibrillated fibre masses stain brilliant yellow with picro-carmine, and there can be no doubt that the fibres of the secretion are ultimately derived from the fibre masses of the club cells.

The earliest stages in the development of the club cells will be more conveniently considered in connection with the description of the sections.

In addition to the club cells the other cell forms found in teased preparations merit a short description here.

These are—(ii.) *Ordinary epidermic cells;* (iii.) *Goblet cells;* (iv.) *Connective tissue cells,* (*a.*) Pigmented, (*b.*) Unpigmented; (v.) Small round cells to which the name of "*fibroblasts*" will be given.

(ii.) *Ordinary epidermic cells.*—These vary much in shape and size according to the depth in the epidermis from which they are taken (as shown by comparison with sections). Illustrations of the various forms will be found in fig. 26, Plate 32.

Cells coming from the region between the stalks of the club cells are laterally compressed with much elongated nuclei (*a*, *b*, *c*, and *l*), while the more superficial cells are polyhedral or rounded with large rounded nuclei (*h* and *i*). It is only upon cells of the latter shape that "prickles" are distinct. Bi-nucleated cells are frequent, but here again I have failed to find mitotic figures (*f*, *g*, *j*, and *k*).*

* January, 1894. In *Petromyzon*, F. E. SCHULZE (6) figures (taf. 8, fig. 2*b*) one of the surface epidermic cells with porous cuticular border, in a stage of transformation into a goblet cell, and W. B. HARDY (35) has described a secretion of granules from the same cells in the Ammocœte larva. In *Myxine* I find several rows of secreting goblets at the surface. In the Eel, as will be evident from the plates, and as I have found by treating the fish with dilute methylene blue solution, there is no evidence of a secretory process in the surface cells of the epidermis.

(iii.) *Goblet cells.*—The full account of these will be found in the description of sections. The cells in teased preparations from macerations in RANVIER'S alcohol are usually swollen and ruptured, presenting an empty theca with wide or narrow stoma, some remnants of protoplasm holding the nucleus lying at the base (fig. 27, Plate 32).

(iv.) *Connective tissue cells.*—That cells of mesoblastic origin may find their way into the epidermis of certain animals is well known. LEYDIG (29) first drew attention to the presence of pigmented connective tissue cells in the epidermis of Reptiles, Fish, and Annulates (*Piscicola*). F. E. SCHULZE (6) described such structures in the epidermis of Eel, Tench, Sturgeon, Silurus, and Ruff. LEYDIG (30) again has shown the presence of such cells among the epidermic cells of Gasteropods, and later (10) demonstrated that "Strahlenzellen," *quite devoid of pigment*, could also occur in the epidermis of Fish (*Cyprinus carassius*). In the same paper he figures (fig. 32, Plate 9) contractile chromatophores from the epidermis of *Pelobates fuscus*. ZIMMERMANN (31) too has described the occurrence of chromatophores among the epidermic cells of the Frog and larval Salamander and their division (mitotic) in the latter.

The pigmented connective tissue cells (chromatophores) of the epidermis of the Eel send their much ramified processes among the inter-epithelial spaces, and are evidently capable of locomotion, since in sections one finds them at various levels, and present in varying number at different spots. They are extremely rare in the epidermis of hybernating specimens. Since these cells break easily in teasing, one only sees their form to advantage in sections, and those figured in fig. 24, Plate 32, are from such preparations.

The non-pigmented connective tissue cells, which LEYDIG has shown may exist among the epidermic cells of Fish, are very abundant in the case of the Eel; indeed, a regular network of these cells appears to exist in the lower layers of the epidermis.

The processes of these cells are of extreme fineness and great length in many cases, and it is these processes that form a felt work surrounding especially the stalks of the club cells.

Between these fully developed, non pigmented connective tissue cells and the fifth kind of cell to be described (fibroblast) a complete series of forms can easily be traced (see fig. 25, Plate 32).

(v.) *Fibroblasts.*—The cells to which I have given this name are without doubt identical with the small rounded cells resembling lymphocytes described by LANGERHANS (7), LIST (13), and FRITSCH (12), already alluded to in the introduction to this paper. The total cell measures only 4 μ to 5 μ, has a nucleus extremely rich in chromatin, and a barely distinguishable rind of protoplasm externally. A few of the isolated cells are figured in the upper left-hand corner of fig. 25. These cells are frequently found dividing, and mitotic figures are easily recognized in their nuclei. As mentioned above, transitional forms between these cells and the unpigmented connective tissue cells are readily found, the change being produced by a simple growth of the protoplasm out into processes, usually commencing on one side first.

The richness in chromatin of the nuclei of these fibroblasts is one of their most marked features, and the nuclei of the developed connective tissue cells have the same characteristic, so that they are very conspicuous in sections (see figs. 5 and 13, Plate 30 ; also Photo E, Plate 33). The origin of these cells will be considered in the description of sections.

(β.) *Sections of Normal Epidermis.*

It is only in sections that the origin of the elements differentiated in teased preparations can be adequately traced. The sections have been cut in planes both vertical and parallel to the surface of the skin. That the sections in the former direction were not oblique was judged at once by observing the appearance of the conical palisade cells of the lowest layer.

The elements requiring description are :—

(a.) The palisade cells and their immediate descendants.
(b.) The club cells at various levels.
(c.) The goblet cells.
(d.) The fibroblasts.
(e.) The formed connective tissue elements.

(α.) *The Palisade Cells.*

Situated upon a basement membrane of some two or three micro-millimetres in thickness, separating the epidermis from the corium, is a single layer of conical cells, whose apices are often prolonged for some distance between the cells of the superjacent layers. The height of these cells is very variable (8 μ to 35 μ) ; the basal breadth is from 4 μ to 8 μ (see figs. 2, 5, 8, &c., Plate 30 ; also Photo A, Plate 33).

The body of the cell stains brownish-yellow with FLEMMING'S fluid, the externally directed process being usually more deeply tinted.

The nuclei are oval, with long axis coinciding with that of the cell, "vesicular," with distinct nuclear membrane, and, as a rule, rather poor in chromatin. In the resting state these nuclei measure 3 μ to 12 μ in length and 1·5 μ to 4 μ in breadth.

Evidence of nuclear division is common (fig. 7, Plate 30), but here again I have never seen mitotic figures. As regards the plane of division of the cell, it appears to be more often parallel to the long axis of the cell than transverse, so that dividing nuclei are most clearly seen in sections cut parallel to the surface of the skin. Divisions at right angles to the long axis are, however, not infrequent.

The descendants of these cells are: (1) *the club cells;* (2) *the goblet cells;* (3) *the ordinary epidermic cells.*

(1.) *Origin of Club Cells from Palisade Cells.*—In transverse sections across the long axis of the palisade cells it is often evident that a lateral bulging of the cell

body occurs before division takes place. This lateral enlargement differs in staining reaction and appearance from the rest of the cell body. In material hardened in FLEMMING'S fluid and stained with saffranine, this differentiated portion appears homogeneous and stained brownish-yellow, while the rest of the cell body is faintly granular, and either lighter yellow or stained pink by the saffranine (see fig. 7, Plate 30).

This lateral enlargement forms the body of the young club cell, and having received its nucleus from the dividing palisade cell, it commences to grow towards the surface.

As a consequence of this mode of origin, one often finds in vertical sections of early stages that the slender lower end of the young club cell is wedged in between two palisade cells, and still in contact with the basement membrane (see fig. 2, *a*, *b*, *c*, and *f*, Plate 30). Though the method of origin above described is by far the most common, it is also possible for club cells to arise by the method of division of the palisade cells at right angles to their long axis (see again fig. 2, *e*, *g*, and *h*, Plate 30). In this case the pointed process of the palisade cell grows out, and at the same time undergoes the same change as occurs in the formation of the lateral enlargement in the first method, becoming homogeneous and staining browner than the rest of the cell body with FLEMMING'S fluid.

The further development of the club cells will be followed under (*b*) of this section.

(2.) *Origin of Goblet Cells from the Palisade Cells.*—A young goblet is always distinguishable from a young club cell in this, that the upper part of the cell instead of being homogeneous and brown in FLEMMING'S fluid and saffranine preparations, is finely granulated and stained red. In fact, the transformation of some of the protoplasm into mucigen occurs before the cell has been constricted off from the palisade cell that gives it birth (fig. 9*a*, 1, 2, and 4, Plate 30).

The nucleus too, in this early stage, shows a far more distinct nucleolus as a rule than that of the young club cell, and this large nucleolus is a feature in the nucleus of the fully developed goblet cell. In the origin of a goblet from a palisade cell, the rule is for the palisade cell to divide at right angles to its long axis, but origin by division parallel to the long axis also occurs. Both of these methods are illustrated in figs. 9*a* and *b*, Plate 30.

The method of origin of the goblet cells of the Fish epidermis has, I find, been variously described by previous observers.

F. E. SCHULZE (6), who investigated thirteen Teleostean genera, including the Eel, and also *Accipenser* and *Petromyzon*, considered that the goblet cells arise from the ordinary epidermic cells of the upper and middle layers, and, in *Petromyzon*, he figures (fig. 2B, Plate 8) a surface cell becoming transformed into a goblet. FŒTTINGER (8) confirms SCHULZE'S account for *Petromyzon*, and FRITSCH (12), using *Malapterurus*, also agrees with SCHULZE with reference to the origin from the ordinary epidermic cells. LIST (9, III.), however, in *Cobitis* and *Torpedo* saw the goblets arising in the lowest layers, though he does not exclude the possibility of origin from the upper cells also.

In the Eel, I have never seen any other method of origin than that above described, *i.e.*, direct from the palisade cells, and these small closed goblets can be traced up through the layers above, expanding as they came to maturity, and finally discharging at the surface.

(3.) *Origin of the ordinary Epidermic Cells from the Palisade Cells.*—Dividing palisade cells are often met with, which show neither of the modifications above described. There can be little doubt that the descendants of these are the ordinary epidermic cells, seeing that cells of the character of those found nearer the surface, but elongated from compression between the stalks of the clubs, are found low down, immediately above the palisade cells (see figs. 29 and 30, Plate 32; also fig. 9*b*, 7, Plate 30).

Again, at the edge of the lip, club cells are absent and goblets few, the mass of the epidermis being formed of the ordinary epidermic cells (see fig. 32, Plate 32).

(*b.*) *The Club Cells at Various Levels.*

The young club cell once separated from its mother palisade cell rapidly grows up into the layers above, and undergoes the following process of development :—

A vesicle becomes developed in the neighbourhood of the nucleus. This process may commence even before the club has separated from the palisade layer (see *g* in fig. 2, Plate 30), but the rule is that the vesicle does not develope till later.

The formation of the vesicle is preceded by the development of a marked granularity in the material immediately surrounding the nucleus (fig. 3, Plate 30). This granular material stains as densely with saffranine as the nucleus itself, though, whether it represents extruded nuclear material or not, I am unable to state definitely, though I am inclined to consider it as modified cell body material. The contents of the vesicle are formed from this granular material, and, as the vesicle grows in size, it is easy to see at its borders, in many instances, a layer of the granular material.

As regards the orientation of the vesicle, it may appear at the outer or inner side of the nucleus, or at both simultaneously (fig 28, Plate 32). A distinct nucleolus is visible within the nucleus at this stage. The material within this vesicle is different to the rest of the club body, and, moreover, does not agree in reaction with the material formed in the goblet cells. The reasons for this statement are as follows : the material in the vesicle stains a grey colour with FLEMMING'S fluid, which is in marked contrast to the brownish-yellow, or brown colour, taken by the material of the club body (figs. 5 and 8, Plate 30). In sublimate specimens treated with the Biondi stain, the material of the vesicle takes the methyl green, while the club body takes the acid fuchsin. Again, as regards differences between the contents of the club vesicles and those of the goblet cells. In specimens fixed with FLEMMING'S fluid, saffranine stains the goblets but not the club vesicles (figs. 5 and 8, Plate 30); the same is true of methylene blue. With thionin the goblets stain reddish-violet, but the club vesicles are not stained (fig. 13, Plate 30). In specimens treated with FLEMMING'S fluid, soluble blue is the

only stain with which I have succeeded in staining the contents of the club vesicles (fig. 6*a*, Plate 30). In sublimate or picric acid specimens, however, saffranine, hæmatoxylin, and methyl green will all dye this material.*

The formed vesicle now continues to enlarge, and a curved line is often noticeable across its wall, separating a denser part below from a less dense part above (fig. 8, Plate 30). This is probably related to the differentiation of a membrane for the escape cell, since in later stages the vesicle again looks homogeneous.

The surrounding wall of club cell body material soon now becomes thinned on one side (fig. 6*b*, Plate 30), and either an "escape cell" is distinctly set freeam ongst the upper epidermic cells (fig. 30, Plate 32), or an "escape mass," containing more or less of the club body material in its walls, is found above the remaining fibre mass (fig. 13, Plate 30; Photo A, Plate 33).

During these later stages the contents of the vesicle assume a distinctly granular appearance in Flemming hardened material, which is absent in the younger condition (*cf.* figs. 6*a* and *b*, Plate 30), and the "escape mass" holds even more granular contents, which, however, retain the peculiarity of staining with soluble blue (fig. 6*c*, Plate 30).

Sections through the upper layers of the epidermis, parallel to the surface, show the escape masses with their granular contents and wall of club body material remarkably clearly. It will be noticeable from a glance at fig. 6*b*, Plate 30, and fig. 30, Plate 33, that the substance constituting what I have termed the "fibre mass," darkens with the osmic acid of the FLEMMING'S fluid to a greater extent than the rest of the club body material, and even in the young club cells the apex and neighbouring parts is, at a very early stage, found to be gradually becoming differentiated in this manner, the process extending from the outer towards the inner part.

Finally, the "escape masses" or "cells" come to lie immediately beneath the surface scales, and are extruded, breaking up in the process, for they are never found as such in the slime itself.

As regards the final fate of the "fibre mass," that part of it which does not pass out with an escape mass, may remain for some time in the epidermis (fig. 5, Plate 30), but it also is gradually eliminated, and one often comes across gaps between the surface cells lined by a few fibres (fig. 13, Plate 30, and fig. 33, Plate 32), indicative of the point of extrusion. Whether the fibre mass is merely passively extruded by the pressure of surrounding growing cells, or whether by virtue of its spiral structure, aided by hygroscopic property, it actively takes part in its own elimination, I cannot say for certain, but the appearance presented in fig. 29, Plate 32, and certain considerations in connection with the histological appearances in artificially stimulated skins, would seem to be in favour of the latter hypothesis.

* January, 1894. I find that the contents of the vesicles of the club cells readily give the LILIENFELD and MONTI (33) phosphorus reaction. The material in the goblet cells, on the other hand, remains unstained, obviously on account of the absence of phosphorus from mucin.

(*c.*) *The Goblet Cells.*

The mode of origin of these cells has been considered in § *a* (2). Once free of the palisade cells they appear to be pushed up by the new cells originating below, growing in size as they approach the surface, but remaining completely closed till it is reached. In material hardened in FLEMMING'S fluid the nucleus which lies in a protoplasmic appendix of the theca (LIST'S "befusste Becherzellen") always holds a remarkably large and distinct nucleolus (figs. 8, 9, 12, and 13, Plate 30, also Photo A, Plate 33). The youngest separated goblets measure 8 μ to 10 μ, the oldest surface specimens may be as large as 60 μ. The shape is very variable (figs. 9, 11, and 12, Plate 30) on account of the variable pressure to which they are subjected at different levels in the epidermis.

The contents of the theca appears in the form of distinct granules in the younger goblets if fixed with FLEMMING'S fluid or osmic vapour, but in sublimate and picric acid specimens and even in FLEMMING'S fluid material in the large surface cells one sees the appearance described by LIST (9, III.) of a "filar and interfilar mass."

Since I have only seen this latter condition in the surface cells in osmic vapour and Flemming hardened material, I consider that it is produced by a process of imbibition of water by the contents of the theca when it nears the surface. I agree with LIST in considering that some such imbibitory process accompanied by swelling brings about the rupture of the theca, and one frequently finds the surface scales in the act of being forced up by an expanding mass of extruded mucus, and later stages in which a plug of mucus projects from the stoma (figs. 12*b* and *c*, Plate 30) above the general surface of the epidermis. The staining reactions of the contents of the theca at all stages of development have already been referred to; the thionin reaction (figs. 11 and 13, Plate 30) is perhaps the most noteworthy. In no case have I seen a goblet cell with two nuclei, a fact confirmed by LIST (9, III.) in the case of the epidermis of Fish.

Apparently the life of a goblet cell is not ended when it reaches the surface and discharges its first load of mucus. Forms are often found in which the protoplasmic foot appears to be growing up (fig. 12, Plate 30, *c* and *d*, on the right), and again elongated cells filled with granules staining black with osmic acid and devoid of mucigen (fig. 11, Plate 30, on the left), which appear to represent the protoplasmic "foot" of a goblet cell. Furthermore large rounded cells, whose body stains reddish-violet with thionin, while the nucleus stains blue, are sometimes found two or three cells below the surface (fig. 10, Plate 30). I can only regard these cells as regenerating goblet cells, and agree with LIST that in the Fish epidermis, at any rate, a goblet cell is capable of more than one discharge of mucus.

(*d.*) *The Fibroblasts.*

These small round cells, already referred to in the description of the teased preparations, are a very marked element in sections on account of the intensity with

which their nuclei stain with most dyes. They are most numerous in the intervals between the apices of the palisade cells, where they often occur in little masses. As one passes towards the surface the relative quantity of these cells diminishes, and in the lower phases of secretory action I have not found them amongst the upper epidermic structures, though in stimulated Fish it may be otherwise. The nuclei in the lower collections of these cells very frequently present mitotic figures (fig. 5, Plate 30), and there can be no doubt that local multiplication occurs.

As regards the origin of these cells, I agree with LIST (13) that they are wandering cells, for not only do I find exactly similar cells between the fibres of the corium as he did in *Cobitis*, but I note them passing through the basement membrane and between the palisade cells, as is very evident in Photo E, Plate 33. The function and fate of these cells is considered in the next paragraph.

(*e.*) *The Formed Connective Tissue Elements.*

These, as already stated on p. 331, are of two kinds, pigmented and non-pigmented.

The pigment cells require no description here as they have already been referred to under (α) of this section, p. 331. They may reach very near to the surface as is evident in fig. 10, Plate 30, but their number is very variable in different specimens.

The non-pigmented connective tissue cells are difficult to distinguish in well-preserved specimens where no shrinkage has occurred; one, at most, sees a fibrous appearance between the other elements, especially in the lower layers of the epidermis. If, however, one observes sections of material in which the club cells have undergone shrinkage (this often occurs in picric acid hardened skins) a complete supporting net formed of the branches of connective tissue cells becomes evident. Photo F, Plate 33, is from a section taken parallel to the surface and the network is there evident. See also fig. 31*b*, Plate 32.

These connective tissue cells appear to arise from the small wandering cells, for, as already mentioned and figured in fig. 25, Plate 32, all stages can be traced between the two forms. I can see no evidence whatever for FRITSCH's hypothesis that they supply the superficial epidermic scales, indeed the demonstration in Photo F, Plate 33, that these cells are of extra-epidermic origin removes the ground from under it at once. Nor again do I see any evidence in the Eel in support of LIST's hypothesis that these cells undergo any degenerative process and pass out as "Schleimkörperchen" in the secretion. Normally, I am convinced that they form the epidermic supporting tissue, and I therefore see no need here of considering any phagocytic theory that might possibly be advanced. I shall again have to refer to these cells in connection with the consideration of the effects of artificial stimulation.

Addendum to § 2.

Nervous structures and connections.—Nerve fibres passing from corium to epidermis are easily observed, and on the afferent side the "becherförmige Sinnesorgane,"

originally described by LEYDIG (1, 10, 29, p. 84), are often met with, being especially clear in the lip (fig. 33, Plate 32). As regards, however, a connection between the secretory elements and the nervous system, I have no certain anatomical results to describe; even in gold chloride preparations I have not been able to convince myself.* As will be seen later, however, in connection with the effects of stimulation, there appears to be physiological evidence of such connections.

Variations in distribution of secreting elements.—F. E. SCHULZE (6) observed that the club cells do not exist in *Petromyzon* at the edge of the ventral fin, nor in the lips. In the lip of the Eel I find they are quite absent at the edge (fig. 32, Plate 32) and on the inner surface, though as one passes towards the general surface of the head these cells gradually make their appearance. The edges of the pectoral fins too are devoid of club cells, though they are present upon the rest of the surface of these organs. The goblet cells I find present over the whole surface of the animal, though local increases in their relative amount (and the same is true of the club cells) are often noticeable. The goblets are most numerous on the inner surface of the lip.

§ 3. THE ORIGIN OF THE FORMED ELEMENTS OF THE SLIME.

Having now considered the structure of the epidermis and the mode of development of its elements, it is possible to treat of the origin of the elements of the slime, whose consideration was discontinued at p. 327.

Threads.—That the threads of the slime originate from the "thread masses" of the club cells there can be no doubt.

It has been seen that elongated "thread masses" on the point of escape (fig. 5, Plate 30) can be detected in the epidermis, and that surface gaps lined by fibres (fig. 13, Plate 30, and fig. 33, Plate 32) may also exist. Again the threads of the slime, and the bodies and "thread masses" of the club cells, give the same brilliant yellow reaction with picro-carmine. And finally, in teased preparations from the epidermis of stimulated Eels one often finds "thread masses" distinctly breaking up into threads (figs. 4*a* and *b*, Plate 30).

The club cells then of the epidermis of the Eel are "Fadenzellen" in the sense applied by KÖLLIKER (2) thirty-three years ago to those of the epidermis of *Myxine*, though in the case before us we have no specialized glandular involutions of the epidermis in which the process of thread formation is carried out to its full extent.

The unravelled threads would not easily be differentiated from unwound threads of *Myxine*, and such a specimen as that depicted in fig. 14, Plate 31, is suggestive even of the complexity of the "aufgewickelte Fadenkörper" of JOHANNES MÜLLER from the slime glands of *Myxine*.

* January, 1894. A few preparations were made by the "Golgi method," but, unfortunately, only the "slow process" was employed, and the delicate epidermis was not well preserved by the potassic bichromate without addition of osmic acid. I intend to again investigate the point by the aid of the "rapid method" with osmic acid, which, I believe, will preserve the integrity of the structure.

In this connection I may state that from *Petromyzon fluviatilis* by suitable stimulation I have obtained a thread secretion which is undoubtedly formed from the club cells of the epidermis, and the elements of which give the characteristic reaction with picro-carmine. FŒTTINGER (8) saw the extrusion of the club cells in this Fish, but he does not describe the final stage of thread formation.

I am, therefore, quite unable to accept the more recent account by POGOJEFF (15), as regards the nervous nature of the club cells of *Petromyzon*, and conclude that, in the three cases I have examined, *Myxine*, *Anguilla*, and *Petromyzon*, the greater part of the club cell is eliminated in the forms of threads, which can be detected in the slime.

Nuclei.—As regards the nuclei of the slime, two possible origins present themselves, (1) From the nuclei of the vesicles of the club cells; (2) From the nuclei of the fibroblasts.

Size is, unfortunately, no help in coming to a decision between these two possible sources of origin, though it at once enables one to state that these slime nuclei are not those of epidermic scales, for the latter are far larger than the former.

FŒTTINGER (8) noted in *Petromyzon* that the extruded club cells did not contain a nucleus, and the fact is evident enough in the fibre masses of the cells in the Eel. The nucleus of the original club cell passes away in the escape cell, or mass, and as this is absent as such from the slime, it must break up upon extrusion, and its nucleus be set free.

As regards the other possibility, origin from fibroblasts, I have not observed that, under "normal" conditions, these reach the surface. On the whole, then, I am inclined to consider the slime nuclei as derived from those of the club cells, though, under conditions of severe stimulation, I think an origin from fibroblasts is also possible.

Granules.—The granules of the slime appear to be the granules of the escape cells, or masses, developed from the contents of the vesicles of the club cells. Their general similarity, and the staining reaction with soluble blue and methyl green, together with the similarity of the LILIENFELD and MONTI phosphorus reaction (*v.* footnotes pp. 326 and 345), in the two cases, form, however, the only basis upon which I make this statement. The mucin streaks and nets found in cover-glass preparations of the slime must owe their origin to the extruded contents of the goblet cells.*

§ 4. The Histological Appearances in Artificially Stimulated Skins.

The details of the process of secretion, so far deduced mainly from the examination of the slime and the variations in appearance of the elements of the "normal" skin, are confirmed, and to some extent supplemented, when the skins of animals submitted to artificial stimulation are investigated.

Summer Fish were used, as a rule, since these are quicker to react to irritation than

* January, 1894. I have failed to find evidence of the presence of any proteolytic ferment, similar to that extracted by Miss ALCOCK (36), from the skin of the Ammocœte larva, in the skin of the Eel.

those obtained in winter, and stimulated skins were obtained in three ways, (1) The pithed animal was suspended in a vessel filled with the vapour of chloroform; (2) The pithed animal was subjected to faradization; (3) An animal, not rendered motionless by the first act of pithing, was allowed to writhe and slime ("mechanical stimulation").

Of these three methods, the first is by far the most efficacious for the production of the highest phase of excitation; indeed, the secretory changes within the epidermis are almost "volcanic" in character, and the structure becomes so loosened that successful histological work requires great care. Since, however, a narcotic action of the chloroform vapour on the elements of the epidermis must finally supervene, this method is not so suitable for observation of the effects of prolonged stimulation as the second. In the third method, one generally finds that secretory action is less marked than in the other cases, and it is liable to be localized to various parts of the surface.

Both teased or cover-glass preparations and sections of stimulated skins have been studied.

(*a.*) *Teased Preparations of Stimulated Skins.*

These have been prepared from chloroform vapour stimulated animals. As one would expect, such preparations differ from the normal mainly in this, that very few normal club cells with closed vesicle are to be found; on the other hand, the field teems with "fibre masses" in various stages of disintegration into slime fibres. Such forms as are figured in figs. 4*a* and *b*, Plate 30, are the rule.

At the same time the number of extruded nuclei is generally very great.

The "escape cells," so abundant in the teased preparations of the normal winter Eels, are conspicuous by their absence in similar preparations from stimulated skins. The process of development of the fibre mass from the club cell and the setting free of the contents of the vesicle is far more energetic than in the normal slow secretory process, and instead of a slow "shelling out" of the vesicle and its contents, an almost explosive disruption of the structure appears to occur in stimulation by chloroform vapour.

One must, however, turn to the sections in order to see how energetic this secretory action may be.

(*b.*) *Sections of Stimulated Skins.*

(1.) *Chloroform Vapour Method.*

As above mentioned, the epidermis of a chloroform vapour stimulated Eel becomes loosened in texture. This loosening is dependent upon three changes:—(α) The eruptive production of spiral fibre masses from the club cells within the epidermis; (β) the production and swelling of goblet cells; (γ) the passage of numbers of fibroblasts into the epidermis.

(α.) *The Changes in the Club Cells upon Stimulation.*—A section through a chloroform vapour stimulated skin at once reveals the fact that few, if any, of the club

cells remain in their normal condition. Curling fibre masses (fig. 36, Plate 32, and Photo C, Plate 33), with granular matter round their upper ends, have now taken the place of club cells with closed vesicles. There can be no doubt that the process of production of the spiral fibre masses from the normal club cells has been the result of the excitation, and instead of the process occurring only in the more developed clubs nearer the surface, it has occurred in the lower layers to almost equal extent. If the excitation has not been severe the surface epidermis may still remain intact (fig. 36, Plate 32, and Photo C, Plate 33), but should it be intense, this eruptive uncoiling of fibre masses may lift the surface cells, with a general disintegration of the epidermis as a resultant. A magnificent example of this will be found in Photo B, Plate 33, which should be contrasted with that of a normal skin in the lowest phase of secretory activity presented in Photo A of the same plate.

The question at once arises: Is this action of chloroform vapour a direct one upon the club cells? or, Is it a reflex action demanding nervous ties with the cord?

The answer is certainly to be given in favour of the latter query, for the following reason, that *exposure of exsected skin to the action of chloroform vapour does not produce the effect.*

There is never any evidence of "an eruption" when the vapour is allowed to act upon the removed skin. Fig. 35, Plate 32, is from a piece of removed skin subjected to chloroform vapour for about fifteen minutes, and is to be contrasted with fig. 36 of the same plate, where the animal with intact spinal cord was stimulated by the vapour. There is, then, physiological evidence of nervous connections for the club cells, though I have not as yet been able to convince myself of the fact anatomically.

It should here be noted that in former experiments upon the electromotive properties of the skin of the Eel (34) I found that exposure of exsected skin to the action of chloroform vapour caused a fall of potential, and I believe that the direct action upon the protoplasm of the secretory elements is narcotic, though in early stages one may have a reflex excitatory effect in the animal with intact cord.

In this reflex stimulation of the club cells it is apparently only the formed cells that react to the excitation; at any rate I have seen no evidence of chloroform excitation leading to a rapid production of new individuals from the palisade cells. Yet it is difficult to imagine how any nerve connection with the club cells can be retained after they have been set free from the palisade layer. Possibly some conducting protoplasmic connection, that has escaped me, is retained between the palisade cells and the club cells, the nerve connection being solely with the former.*

(*β.*) *The Production of Goblet Cells.*—At the same time that this extraordinary change is produced in the club cells, a great production of goblet cells also appears to take place upon stimulation with chloroform vapour. Normally a small rounded goblet cell occurs here and there above the apices of the palisade cells. After stimulation a

* Or it may be that we have to deal only with *contiguity* of nerve fibres to irritable structures.

dense crop is usually found above the palisade layer (fig. 36, Plate 32), generally giving evidence of being subjected to pressure by their elongated form.

At the same time the older goblets in the upper layers became swollen, and even in osmic and Flemming hardened material have lost their granularity, which is replaced by a network.

This change is apparently due to a local action of the vapour, for I have not generally found much difference in number of goblet cells between the exsected skins and those stimulated on the animal. The paucity of goblets in fig. 35, Plate 32, is rather exceptional.

(γ.) *The Passage of Fibroblasts into the Epidermis.*—The quantity of fibroblasts in the lower layers of stimulated skins is one of their most marked features. This is clear in fig. 36, Plate 32.

This change can, of course, only occur so long as the skin is on the animal, and, consequently, exsected skins subjected to chloroform vapour do not show this peculiarity.

That these cells are really exuded leucocytes is forced upon one when one observes transverse sections of blood-vessels. Capillaries lie some 5 μ or 10 μ below the basement membrane, and in stimulated skins it is easy to trace the leucocytes from the vessels towards the epidermis. The extent to which diapedesis may occur is depicted in fig. 34, Plate 32, from a section across the pectoral fin.

In the rapid processes of reflex secretion evoked by chloroform vapour, it would appear that all these extruded cells are not converted into connective tissue corpuscles, for one finds them unaltered in the upper layers of the epidermis, and sometimes large masses of small cells, 5 μ to 6 μ in diameter, are found lying upon the surface of the stimulated skin. A good instance is seen in Photo B, Plate 33.

It is, of course, possible that many of these nuclei are from extruded club cell vesicles, but since I find a distinct cell body round most, I am inclined to consider them as undeveloped fibroblasts.

(2.) *Stimulation by Faradization.*

Faradization does not appear to act as such a strong stimulus as chloroform vapour, but, since it can have no narcotic after-effect, can be used for observing the effects of a long period of moderate excitation, in contrast to the violent but brief reflex action of chloroform. Prolonged faradization supplies skins without a trace of surface epidermic cells, though one finds that multiplication in the lower layers is very active. Again, young club cells are frequent in the lower layers, but are usually directly extruded as such, vesicle and all, so that in Eels faradized in air, a collection of extruded club cells is found upon the surface, many giving signs of subsequent disintegration. An illustration of this is seen in Photo D, Plate 33. FŒTTINGER (8) has already figured these extruded club cells in *Petromyzon* (see figs. 10, 11, and 12, Plate 31, of his paper). The nuclei of the palisade cells also exhibit signs of division to a very marked extent.

No marked production of goblet cells, on the other hand, is evident in faradized skins. Fibroblasts are, however, very numerous in the lower layers (Photo D, Plate 33), and extravasated red corpuscles are often found among the fibres of the corium.

(3.) *Mechanical Stimulation.*

The changes met with in the skins of Eels that have writhed in the process of capture are, as would be expected, less marked and often localized.

They have already been incidentally referred to in previous pages, since such moderate conditions of stimulation could scarcely be separated from the account of the slow process of normal secretion. Figures in illustration are given in Plate 30, fig. 5, and Plate 33, fig. 29.

The most marked effect, therefore, of subjecting Eels to artificial stimulation is to cause a rapid production of fibre masses from the club cells. In stimulation by chloroform vapour, this process is almost "volcanic" in its energy, the epidermis being so loosened that slight pressure is sufficient to cause removal of the upper layers. The animal, therefore, actually leaves a considerable part of its epidermis behind when it is gripped, the regenerative palisade layer, however, always remaining. The increase of fibroblasts, which also occurs, is suggestive of the idea that these may act as a temporary protection and support by development into connective tissue corpuscles, during subsequent regeneration of lost elements.

Note upon the Action of Atropine.

Eels are very resistant to intoxication with atropine, and live for days in fairly strong solutions. It is, however, possible to obtain histological effects, which are of some interest and are indicative of a paralytic action of the drug as regards secretion.

The skins of atropinized Eels teem with fibre masses and escape masses, yet there is no evidence of rapid removal of the surface or escape of the secretory elements or their products. Figures from sections of an atropinized Eel, which refused to give any excitatory electrical variation upon faradic excitation (see my former paper, 'Phil. Trans.', vol. 184, B, p. 360), are given in Plate 32, figs. 31*a* and *b*. It would appear that the appearances there seen are to be accounted for in the following manner. At first, the introduction of the animal into a foreign medium has induced a secretory action, but this has soon become paralyzed, so that the fibre masses, &c., first formed are retained in the epidermis. The whole epidermis of such Eels is, too, actually on an average thicker than normal, while in Eels stimulated for long periods (faradization or life in pilocarpine bath), it becomes thinned.

The club cells have not the normal appearance (fig. 31*b*), the material of the head having undergone change into a granular material instead of the ordinary more clearly defined fibre mass material. The club in this figure has developed to the full, yet it has never left the palisade layer. Fibroblasts too, are exceedingly rare, while

on the other hand, ordinary epidermic cells are found compactly set right down to the palisade cells.

The skins certainly have the appearance of having been stimulated to secrete rapidly, but the process subsequently paralyzed.

Conclusion.

It is evident from the foregoing account that the secretory process in the skin of the Eel presents several points of peculiarity and interest. The demonstration of a secretion of threads from the club cells points to a similarity of function of these cells in such otherwise widely separate Fish as the Eel and *Myxine*. The process is certainly better developed in the latter animal, and it has, moreover, the advantage of special epidermic involutions devoted to manufacture and store of threads; yet in both, the whole club cell is cast off and mainly in the form of threads.

The fact that *Petromyzon* produces a thread secretion was to be expected, but had not been before clearly demonstrated, though the extrusion of the club cells was known.

What relation, if any, these thread-producing epidermic cells in Fish have to those of larval Amphibia described by Leydig (10) and Eberth (11), it is not my place to speculate upon. As regards the function of these threads in the slime of the Eel, I have already stated my opinion that they are of value in causing the slime to adhere to objects with which it is brought in contact. In *Myxine*, however, I cannot but think that the cloud of thread-penetrated slime that the animal is capable of discharging, must be of service in aiding it to attack the far more powerful Fish that often become its host.

The physiological process involved in the sudden development of a spiral fibre mass upon stimulation, leading to a veritable upheaval of the epidermis, presents a problem in cell mechanics, at present inscrutable, but of great interest, though the effect in giving the animal the power to loosen the surface of its skin explains the proverbial slipperiness.

The connective-tissue network of the epidermis appears as a special arrangement for consolidating an otherwise extremely labile structure, and the observation that during excitation provision is made for a fresh supply of supporting cells by an inroad of fibroblasts would almost be expected from the nature of the case.

In fine, the following conclusions may be summarized:—

1. The secreting elements of the epidermis of the common Eel consist of goblet cells and club cells, both direct descendants of the cells of the palisade layer. The former supply a mucin, the latter threads and a material appearing as fine granules in the slime.

2. The goblet cells contain mucin granules, and, after reaching the surface and discharging their load, are capable of undergoing regeneration, by growth of the protoplasmic foot and re-formation of mucin.

3. The threads of the slime resemble those of *Myxine glutinosa*, but are usually of finer texture. As in *Myxine*, they are developed from the club cells, but there are no special glandular involutions of the epidermis. The club cells of *Petromyzon fluviatilis* also supply slime threads.

4. The granular material of the slime is the contents of vesicular spaces developed in the club cells in the immediate neighbourhood of their nuclei, and is set free enclosed in a lattice work developed by vacuolation of the surrounding material and finally extruded, carrying with it the original nucleus of the club cell.

5. The remainder of the club cell after extrusion of its vesicle and nucleus becomes a spirally coiled fibre, which finally breaks up into the fine fibrils of the slime.

6. Severe stimulation, especially by the vapour of chloroform, applied to the intact animal, causes so sudden a development of the coiled fibres from the club cells that the surface of the epidermis is thrown off and the secretory products set free *en masse*. This process is of reflex nature, for similar excitation applied to excised skin is without effect.

7. A system of connective tissue cells, distinct from chromatophores, exists in the epidermis developed from cells which are direct descendants of leucocytes, and which can be traced from the bloodvessels of the corium through the basement membrane of the epidermis. The number of these wandering cells in the epidermis is greatly increased by stimulation, probably with a view to providing subsequent support to the secretory elements during regeneration.

Appendix.

Histological Procedure.

Isolation of Elements.—The difficulty of isolation of the fresh elements of the epidermis, on account of the viscous nature of the structure, compelled me to resort to maceration. Ranvier's "third part" alcohol was found to deform the elements less than any other dissociating liquid, and was used throughout.

The skin was placed for 24 to 48 hours in this fluid, and then either shaken in water to separate the elements, or cover-glass preparations of the softened epidermis were made in the usual manner. In preparing the elements of the slime the cover-glass method was used, except in cases where the flocks of secreted matter rising from an Eel in a bath were collected, in which cases the material was placed at once in $\frac{1}{10}$ per cent. osmic acid solution, and, after a few days, teased in dilute glycerine. The dry cover-glass preparations were either stained directly with picro-carmine, Biondi stain, methylene blue, or soluble blue, or, if to be stained with thionin, first treated with corrosive sublimate. The macerated material not made into cover-glass preparations was either stained by irrigation in small teased masses on the slide, or stained in bulk, and subsequently teased in dilute glycerine. The action of digestive ferments was watched in the hanging drop in an Engelmann's gas chamber placed upon a Schäfer's warm stage kept at 37° C.

Preparation of Material for Sections.

The following fixing reagents have been used from time to time. Osmic vapour. FLEMMING'S fluid. PERENYI'S fluid. Picric acid (saturated aqueous solution). Corrosive sublimate (saturated aqueous solution). Nitric acid, 10 per cent., for 24 hours, followed by osmic acid, 1 per cent., for 24 hours. Equal parts of saturated aqueous corrosive sublimate solution, and 1 per cent. chromic acid solution. Absolute alcohol containing 25 per cent. of glacial acetic acid. In all cases a graduated series of alcohols was subsequently employed.

The best results were obtained with FLEMMING'S fluid and the nitric-osmic method, though the corrosive sublimate preparations were very reliable.

Staining.—This was usually effected in bulk. A good nuclear saffranine from GRÜBLER used in semi-alcoholic solution gave the finest results upon the material fixed in FLEMMING'S fluid and by nitric and osmic acids. Methylene blue was also valuable. The corrosive-hardened material was stained in bulk usually with Biondi stain or hæmatoxylin.

If staining was effected after cutting sections, saffranine, hæmatoxylin, and soluble blue were used for the Flemming fixed material, or thionin after previous treatment of the sections with corrosive sublimate solution. I found the mucin reaction with thionin succeeded as well in the Flemming fixed material as in corrosive fixed epidermis, provided the former was first treated with corrosive sublimate, but, unfortunately, the specimens soon fade. Sections of material fixed with corrosive sublimate were stained with thionin, picro-carmine, Biondi stain, or hæmatoxylin.

In all cases where it was necessary to hold parts together, or where the preservation of the condition of the surface was absolutely necessary (see, for instance, Photos B and D, Plate 33), the sections were cemented to the slide by floating on in 50 per cent. alcohol while still in paraffin, and subsequent drying at 35° C.

The paraffin method, with xylol, has been used throughout for obtaining the sections.

BIBLIOGRAPHY.

1. LEYDIG. "Ueber die Haut einiger Süsswasserfische." 'Zeitsch. f. wissensch. Zoologie,' vol. 3, 1851, p. 1.
2. KÖLLIKER. "Ueber der Inhalt der Schleimsäcke der Myxinoiden und die Epidermis der Neunaugen. 'Würzburger naturwissensch. Zeitsch.,' vol. 1, 1860, p. 1.
3. J. MÜLLER. 'Unters. über die Eingeweide der Fische,' Berlin, 1845, p. 11.
4. MAX SCHULTZE. "Die kolbenförmigen Gebilde in der Haut von *Petromyzon*

und ihr Verhalten im polarisirten Lichte." REICHERT u. DU BOIS-REYMOND'S 'Archiv,' 1861, pp. 228–247, and pp. 281–303.

5. H. MÜLLER. "Bemerkungen über die Epidermis von *Petromyzon*." 'Würzburger naturwissensch. Zeitsch.,' vol. 1, 1864, p. 43.
6. F. E. SCHULZE. "Epithel- und Drüsen-Zellen." 'Arch. f. mik. Anat.,' vol. 3, 1867, p. 137.
7. P. LANGERHANS. Unters. über *Petromyzon planeri*,' Freiburg, 1873, pp. 14–23.
8. A. FŒTTINGER. "Recherches sur la structure de l'épiderme des Cyclostomes," &c. 'Bulletin de l'Académie royale de Belgique,' 2me série, vol. 41, 1876, p. 599.
9. LIST. (i.) "Ueber einzellige Drüsen (Becherzellen) in der Oberhaut von *Torpedo marmorata*." 'Zoolog. Anzeiger,' Jahrg. 8, 1885, p. 388.
   (ii.) "Ueber den Bau, die Sekretion, und den Untergang von Drüsenzellen." 'Biolog. Centralblatt,' 1885–86, p. 698.
   (iii.) "Ueber Becherzellen." 'Arch. f. mik. Anat.,' vol. 27, 1886, p. 481.
10. LEYDIG. 'Hautdecke u. Hautsinnesorgane der Fische.' Halle, 1879.
11. EBERTH. "Zur Entwicklung der Gewebe im Schwanze der Froschlarven." 'Arch. f. mik. Anat.,' vol. 2, 1866, p. 499.
12. FRITSCH. 'Die Electrischen Fische.' Erste Abtheil., "*Malapterurus*," 1887. Cap. V., "Die äussere Haut," p. 38.
13. LIST. "Studien an Epithelien. 1. Ueber Wanderzellen im Epithel." 'Arch. f. mik. Anat.,' vol. 25, 1885, p. 264.
14. STOHR. "Ueber Mandeln u. Balgdrüsen." VIRCHOW'S 'Archiv,' vol. 97, 1884.
15. POGOJEFF. "Ueber die Haut des Neunauges." 'Arch. f. mik. Anat.,' vol. 34, 1889, p. 106.
16. BLOMFIELD. "The Thread-cells and Epidermis of *Myxine*." 'Quart. Journ. Microsc. Science,' vol. 22, 1882, p. 355.
17. EISIG. 'Fauna u. Flora des Golfes von Neapel.' XVI. Monographie, "Die Capitelliden," vol. 1.
18. MÖBIUS. "Ueber die Eigenschaften u. den Ursprung der Schleimfaden des Seestichlingsnestes." 'Arch. f. mik. Anat.,' vol. 25, 1885, p. 554.
19. HOYER. "Ueber den Nachweis des Mucins in Geweben mittelst der Färbemethode." 'Arch. f. mik. Anat.,' vol. 36, 1890, p. 310.
20. HEIDENHAIN. "Beiträge z. Histologie u. Physiologie d. Dünndarmschleimhaut." PFLÜGER'S 'Archiv,' vol. 43, 1888. Supplementheft.
21. PANETH. "Ueber die secernirenden Zellen des Dünndarmepithels." 'Arch. f. mik. Anat.,' vol. 31, 1888, p. 113.
22. LEYDIG. 'Anatomisch-histologische Unters. über Fische u. Reptilien,' Berlin, 1853, p. 107.
23. LANGERHANS. "Ueber die Haut der Larve von *Salamandra maculosa*." 'Arch. f. mik. Anat.,' vol. 9, 1873, p. 745.

24. Flemming. "Beiträge zur Kentniss der Zelle u. ihrer Lebenserscheinungen." Arch. f. mik. Anat.,' vol. 14, 1879, p. 316.
25. Pfitzner. "Die Epidermis der Amphibien." 'Morphologisches Jahrbuch,' vol. 6, 1880, p. 469.
26. Carrière. "Die postembryonale Entwicklung der Epidermis des *Siredon piscifformis*." 'Arch. f. mik. Anat.,' vol. 24, 1885, p. 19.
27. Paulicki. "Ueber die Haut des Axolotls." 'Arch. f. mik. Anat.,' vol. 24, 1885, p. 120.
28. Leydig. 'Zelle u. Gewebe,' Bonn, 1885, p. 89.
29. Leydig. 'Lehrbuch der Histologie,' 1857, pp. 99 and 120.
30. Leydig. "Die Hautdecke u. Schale der Gastropoden," &c. 'Arch. f. Naturgeschichte,' 1876, p. 208.
31. Zimmermann. "Ueber die Theilung der Pigmentzellen, speciell der verästelten intraepithelialen." 'Arch. f. mik. Anat.,' vol. 36, 1890, p. 404.
32. Waymouth Reid. "Mucin Granules of *Myxine*." 'Journal of Physiology,' vol. 14, p. 340.
33. Lilienfeld und Monti. "Ueber die mikro-chemische Localisation des Phosphors in den Geweben." 'Zeitsch. f. Physiologische Chemie,' vol. 17, p. 410.
34. Waymouth Reid. "The Electromotive Properties of the Skin of the Common Eel. 'Phil. Trans.,' vol. 184 (1893), B., pp. 335–365.
35. Hardy. "On the Reaction of certain Cell-Granules with Methylene-Blue." 'Proc. Cambridge Phil. Soc.,' vol. 7, part 5, 1891, p. 258.
36. Alcock. "The Digestive Processes of Ammocœtes." 'Proc. Cambridge Phil. Soc.,' vol. 7, part 5, 1891, p. 252.
37. Waymouth Reid. "Chemical Note on the Secretion of *Myxine glutinosa*." 'Journal of Physiology,' vol. 15, p. 488.

Description of Plates 30-33.

Zeiss' apochromatic objectives and oculars have been used throughout, and the drawings have, without exception, been executed with the Abbe camera lucida. The photographs in Plate 33 were taken with the same system of objectives supplied with projection oculars, the source of light being a 3000-candle power arc lamp. My thanks are due to Professor Claxton Fidler, of the Engineering Department of University College, Dundee, for his kindness in supplying me with current for the lamp.

PLATE 30.

Fig. 1. × 600. From a "cover-glass preparation" of the slime of a "normal" Eel, stained with picro-carmine. The characteristic yellow reaction of the threads of the slime is shown, also relative size of extruded nuclei and granules. An extruded goblet cell is present on the left.

Fig. 2. × 600. Origin of club cells from palisade cells. FLEMMING'S fluid and saffranine; *a*, *b*, *c*, and *f* show the method of origin by lateral division of the palisade cell at right angles to its longer axis. The darkening of the apex of the young club cell by the FLEMMING'S fluid is visible in all cases.

Fig. 3. × 600. Stage in development of club cells previous to the formation of the vesicle. Fixing and staining as in fig. 2. Note the granular matter densely stained with the saffranine, in the region of the nucleus.

Fig. 4*a* and *b*. × 600. Two stages in the development of the slime fibres from the "fibre-masses" of the club cells. From a "cover-glass" preparation of an epidermis stimulated by chloroform vapour. RANVIER'S alcohol and picro-carmine. The same yellow reaction is evident as in the formed fibres of the slime (fig. 1) in both cases, and in *b* the breaking up into the finest fibres is seen.

Fig. 5. × 600. Developed "fibre-masses" in process of extrusion. From a winter Eel that had struggled during killing. FLEMMING'S fluid and saffranine. Note the dark coloration of the fully-formed "fibre-mass," and compare with that of the apices of the club cells still possessing a vesicle. Also, the absence of saffranine staining of the contents of the club vesicle should be contrasted with the marked coloration of the contents of the goblet cells. Note also a considerable number of fibroblasts in the lower layers, many of which exhibit mitotic figures.

Fig. 6*a*, *b*, and *c*. × 600. Similarity in staining reaction of the contents of the club vesicle at early and later stages with that of the "escape mass." Winter Eel, FLEMMING'S fluid and soluble blue. The contents of the fully developed vesicle and "escape mass" are distinctly granular, that of the young vesicle is homogeneous.

Fig. 7. × 600. Division of palisade cells. FLEMMING'S fluid and saffranine. The upper examples are from sections parallel to the surface of the skin, the lower are cut at right angles to the surface. In the upper note the lateral bulging, staining yellowish-brown, and indicative of the commencement of differentiation of the club cell body material. In the lower, the deeper staining of the apices of the conical palisade cells is noticeable. No mitotic figures are to be seen.

Fig. 8. × 600. Medium stage in the development of a club cell and a goblet cell. FLEMMING'S fluid and saffranine. In the vesicle of the club cell, note a curved line, separating a denser lower part from a less dense upper part. This line passes upwards, as development proceeds, and probably marks the edge of the membrane of the "escape cell," which is becoming differentiated from the wall of the club. Note the "foot" and marked nucleolus of the goblet cell, its granular contents, and difference in staining reaction

to those of the club vesicle. The dividing palisade cell immediately below the goblet cell is giving rise to an ordinary epidermic cell.

Fig. 9*a* and *b*. × 600. Origin of goblet cells from palisade cells. FLEMMING's fluid and saffranine.

9*a*. The transformation of some of the protoplasm of the young goblet cell into a saffranine staining mucigen, even before the new cell has parted from its mother, is seen in 1, 2, and 4, and should be contrasted with the early stages and staining reactions of the young club cells in fig. 2. 2 is a case of origin by lateral division; 1 and 4, by transverse division of the palisade cell, though in the latter case the two nuclei of the palisade cell show that a lateral division will take place shortly. 3 shows how early the characteristic shape is attained in many cases.

9*b*. 6 shows that the characteristic shape may be developed even before separation from the parent, and also (as, too, in 8) the great length to which the outwardly directed process of the palisade cell may reach. In 7, a goblet cell is being thrust upwards by an ordinary epidermic cell with characteristic elongated nucleus.

Fig. 10. × 600. Regenerating goblet cell (re-formation of mucigen). FLEMMING's fluid. Thionin, after treatment with corrosive sublimate. Winter Eel. Note the characteristic mucin reaction with thionin in the protoplasm of this cell, and contrast with the ordinary blue colour of thionin taken by the nucleus of this cell and those of the surrounding epidermic cells. A wandering pigment cell is also to be seen.

Fig. 11. × 600. Goblet cells in earlier stages of regeneration than that in fig. 10. Preparation as in case of fig. 10. The cell on the left consists of the protoplasmic "foot" alone, containing granules staining black with osmic acid. The theca, with its load of mucigen, has disappeared, but no new supply has been yet formed, as in fig. 10. The two cells on the right indicate stages in the growth of the protoplasm of the "foot," in cases where the first load of mucigen has not been completely discharged. The darkened granules are again seen here; they are never seen in the "foot" of ripe cells (*cf.* fig. 12*a*). The nucleolus is, as a rule, absent in the regenerating cells.

Fig. 12. × 600. Ripe, discharging, and regenerating (earliest stages) goblet cells. FLEMMING's fluid. *a* is stained with methylene blue; *b*, *c*, and *d* with hæmatoxylin. In *b* and *c*, which are discharging, the stoma of the theca and the projecting "pfropf" of mucus are seen. *a* is a cell ripe for discharge, the theca still being intact. *d* is an intermediate stage in regeneration between the cells on the extreme right and left of fig. 11.

Fig. 13. × 435. Section of winter Eel killed "instantaneously," and in lowest phase of secretory activity. FLEMMING's fluid. Thionin, after treatment with

corrosive sublimate. Note the reddish-violet reaction of the contents of the goblet cells, and compare with the absence of staining of the contents of the club vesicles and the blue colour of the nuclei throughout. A "fibre mass," tending to be spiral, is seen on the left, and a point of escape, lined by a few fibrils, is seen above. A thick layer (7 or 8 cells) of superficial epidermic cells is present.

PLATE 31.

Fig. 14. × 600. Convoluted fibre, discharged by an Eel placed in a bath of pilocarpine solution. $\frac{1}{10}$-per cent. osmic. Dilute glycerine.

Fig. 15*a* and *b*. × 600. Club cells with dividing nuclei. Winter Eel. RANVIER'S "third part" alcohol. Carmine. Dilute glycerine. Note the lattice work in the wall of the vesicle and the "prickles" upon the outer surface of the body of the cell.

Fig. 16*a* and *b*. × 600. Club cells with two vesicles, each holding a nucleus. Preparation as in fig. 15. In *a*, note the granular core passing down the stalk from the vesicle.

Fig. 17*a*, *b*, and *c*. Extrusion of the "escape cell" from the club. *a* and *c*, × 435. *b*, × 600. Preparation as in fig. 15. The lattice work in the wall of the "escape cell" is especially clear in *b*. In *c*, the presence of a second nucleus indicates the possibility of formation of a second "escape cell."

Fig. 18*a* and *b*. Development of a coiled "fibre mass" without the formation of a definite "escape cell." (The remainder of the vesicle is in such cases referred to as an "escape mass" in the text.) *a*, × 435. *b*, × 600. Preparation as in fig. 15.

Fig. 19*a* and *b*. Secondary vacuolation of "fibre mass." *a*, × 435. *b*, × 600. Preparation as in fig. 15. Note granularity in secondary vacuolation spaces in *a*, similar to that occurring in the stalk in figs. 15, 16, and 17.

Fig. 20*a*, *b*, *c*, and *d*. × 600. Stages in the vacuolation of the club body material, leading up to the freeing of the "fibre mass." Preparation as in fig. 15. The vacuolation and formation of a lattice work, filled with granular matter, is seen commencing in *a*, and further developed in *b*. In *c*, this granular material has become freed in the process of maceration, so that the trellis of the residual unaltered club head material is very distinct, In *d*, the "fibre mass" is free. The "prickles" noticeable in figs. 15, 16, 17, and 18 are retained upon the lower end of the developed "fibre mass."

Fig 21*a* and *b*. × 600. Large isolated "escape cells." Preparation as in fig. 15. In this form, the greater part of the club body material is converted into granular material, and the "fibre mass" remains attached as a convoluted coarse filament.

Fig. 22*a*, *b*, and *c*. × 600. Isolated "escape cells." Preparation as in fig. 15. In *a* some of the trellis produced in the vacuolation of the club head is still adherent; *b* has no thick wall of club head material, and is an intermediate form between an "escape cell" and "escape mass." In *c* the thick wall is complete only on one side.

Fig. 23*a*, *b*, *c*, *d*, *e*, *f*, and *g*. × 600. Preparation as in fig. 15. These figures show the great variation in size and structure of "escape cells" met with in teased preparations; *a* and *b* show the possibility of division of the nucleus; *b*, *c*, and *d* show the nucleus in process of extrusion; *e* and *g* show how extremely small these cells may be in comparison with such forms as fig. 21*a* and *b*; *f* (as also in fig. 22*a*) shows a stout strand of club body material that has been ruptured in the process of "shelling out" of the cell. In all, the outer surface is seen to be beset with spines of the original lattice work—a fact also frequently clear in sections *c*, *f*, fig. 6*c*, Plate 30.

N.B.—All the figures upon this Plate, with the single exception of fig. 14, are from one winter Eel, killed "instantaneously."

PLATE 32.

Fig. 24. × 600. Pigment cells of the epidermis, from a section hardened in FLEMMING'S fluid.

Fig. 25. × 600. Fibroblasts, isolated by RANVIER'S alcohol. Carmine. Glycerine. In passing from left to right, all stages, from the simple lymphocyte-like cell to the small connective tissue cell with long fine processes, are to be observed.

Fig. 26. × 600. Ordinary epidermic cells, from various levels. Preparation as in fig. 25. *a*, *b*, *c*, and *l*, with elongated nuclei, are from the lower layers; *d* and *e*, from a higher level; *h* and *i*, from near the surface; *f*, *g*, *j*, and *k* are dividing cells from near the surface, but lack mitotic figures in their nuclei. It will be noted that the more superficial cells present "prickles."

Fig. 27. × 600. Isolated goblet cell. Preparation as in fig. 25. The plicated wall of the empty theca and the marked nucleolus of the nucleus are to be noted.

Fig. 28. × 600. Formation of the vesicle of the club cells. From sections of material hardened in FLEMMING'S fluid, and stained with saffranine. This condition is presented in the stage following that depicted in fig. 3, Plate 30. The intimate relation of the vesicle to the nucleus is to be noted, as also the granular transformation of the club body material which precedes the enlargement of the vesicle. The vesicle may start on the outer

side of the nucleus *a*, *f*, *g*, and *i*, with granularity only at the opposite pole, or *vice versâ*, *h* and *k*; on the other hand, it is possible for it to arise simultaneously upon both sides, *b*, *c*, *d*, and *e*.

Fig. 29. × 435. An escaping "fibre mass," lifting surface cells. From a winter Eel that had struggled during capture. FLEMMING'S fluid and saffranine.

Fig. 30. × 435. An "escape cell" and related "fibre mass" *in situ*, in a section. Same Eel and preparation as fig. 29.

Fig. 31*a* and *b*. *a*, × 160. *b*, × 600. Appearances in the epidermis of an Eel that had lived two days in a solution of atropine. FLEMMING'S fluid and saffranine. In *a*, "fibre masses" and "escape masses" are seen to exist at all levels of the epidermis, and a peculiar granularity is noticeable round about the upper ends of the "fibre masses." The general appearance of the section is suggestive of an initial secretory activity, which has been subsequently brought to a close by the paralyzing action of the drug. *b* shows the appearance of a club cell still in contact with the palisade layer, which, though developed to the full, has never left its position of origin. A developed fibroblast with long processes is seen ensheathing this club, at the upper part of the figure.

Fig. 32. × 435. Section of outer edge of lip. Picric acid and hæmatoxylin. Note the absence of club cells, presence of a goblet cell, and one of the well-known sense organs.

Fig. 33. × 600. Point of escape of a "fibre mass." From a winter Eel. FLEMMING'S fluid and thionin.

Fig. 34. × 600. Diapedesis. From central vessels of pectoral fin. Picric acid and hæmatoxylin. These extravasated cells are quite indistinguishable from the young fibroblasts found in the lower layers of the epidermis.

Figs. 35 and 36. × 435. Fig. 35 is a section of skin subjected to the action of chloroform vapour *after removal*. Fig. 36 is from the skin of an Eel simply decapitated and then exposed to the vapour. Both were fixed with corrosive sublimate and stained with hæmatoxylin. In fig. 35, most of the club cells are in the earlier stage, with closed vesicle. In fig. 36, only curled "fibre masses" are found, with some granular débris around them. There is also to be noted, in fig. 36, a relative increase in the quantity of goblet cells and fibroblasts in the lower layers. *Cf.* also Photos B and C, Plate 33.

PLATE 33.

The prints on this Plate are Collotypes, direct from untouched negatives.

Photo A. × 320. Epidermis of a "normal" winter Eel. FLEMMING'S fluid and saffranine. Note below the club cells, with closed vesicles, and the

conical palisade cells; above, the free "escape masses." This print represents the appearances of the lowest phase of secretory action.

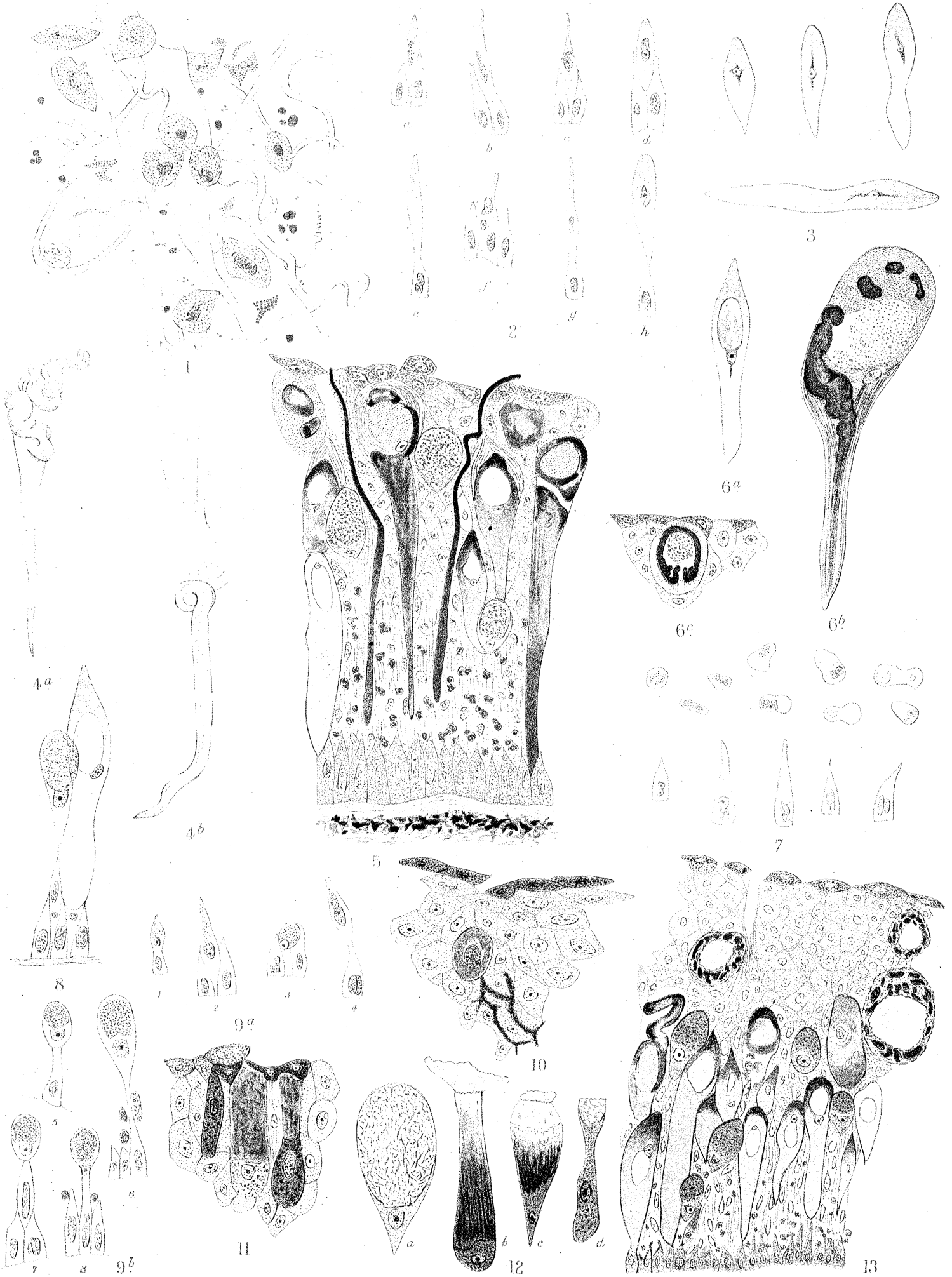
Photo B. × 175. Later stages of stimulation by chloroform vapour. Nitric and osmic acid. Saffranine. Note the "eruption" of fibre masses (darkly stained) causing considerable disruption of the layers of the epidermis. Above is seen a compact mass of small cells (5μ), which are, probably, extruded fibroblasts. A pad of coagulated mucus, containing a few epidermic cells, separates this mass of small cells from the parts below. This print should be contrasted with the appearance of the resting skin seen in Photo A.

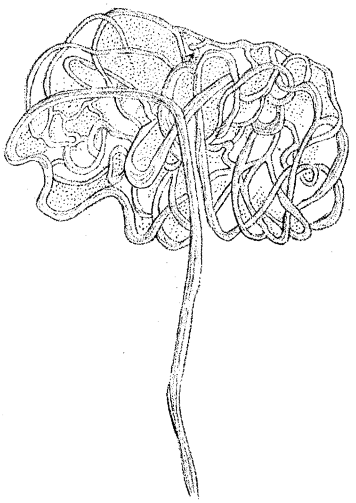
Photo C. × 300. Early stages of stimulation by chloroform vapour. Compare with Photo B. Nitric and osmic acids. Saffranine. A good example of a coiled fibre mass is seen on the right.

Photo D. × 175. Epidermis of a summer Eel faradised for an hour. FLEMMING'S fluid and saffranine. The superficial epidermic cells are absent, and the surface consists of a mass of extruded fibre masses and club cells. An escaping club cell is evident upon the left. Note the large number of fibroblasts in the lower layers.

Photo E. × 900. Passage of fibroblasts from the corium into the epidermis. Picric acid and hæmatoxylin. Fibroblasts can be seen upon the corium side of the basement membrane, one is fixed as it passes through, another between two palisade cells, and others lie free in the epidermis. In two of these latter a protoplasmic process is evident upon one side of the cell. Note the relative sizes of the nuclei of the palisade cells and the fibroblasts.

Photo F. × 450. Connective tissue network in the epidermis. Section parallel to the surface of the skin about midway between surface and basement membrane. Picric acid and saffranine. The bodies of the club cells have shrunk in preparation, and so allow the branching cells to be easily visible.

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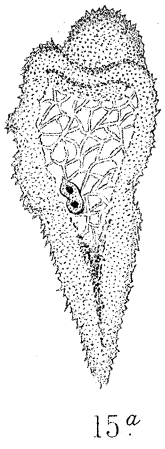
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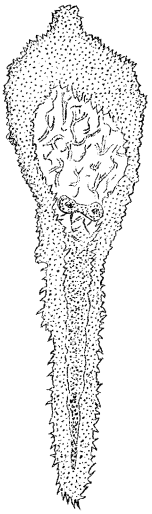
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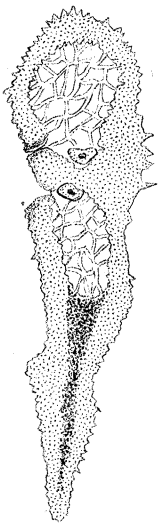
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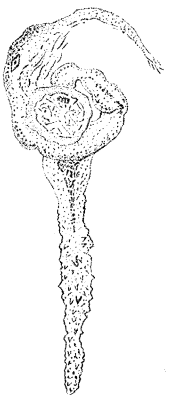
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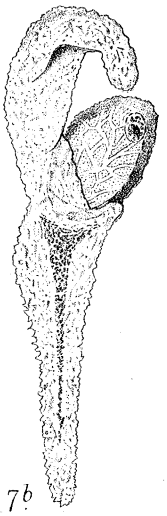
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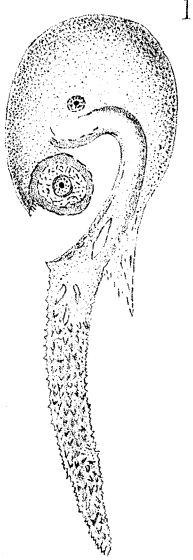
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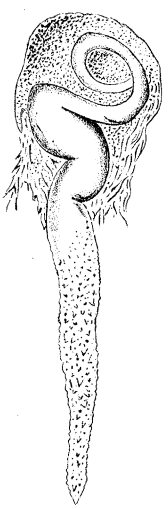
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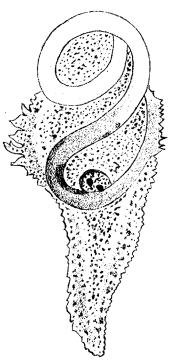
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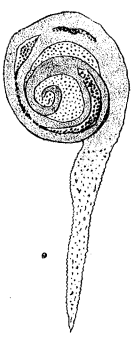
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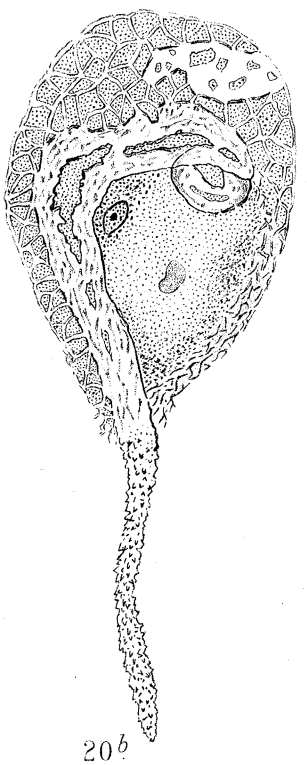
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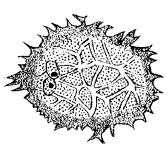
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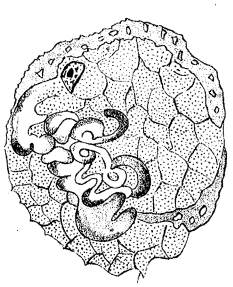
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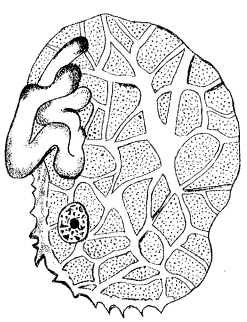
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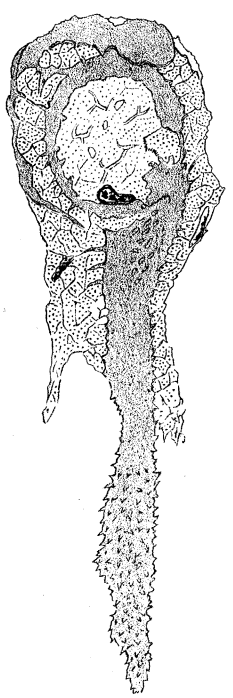
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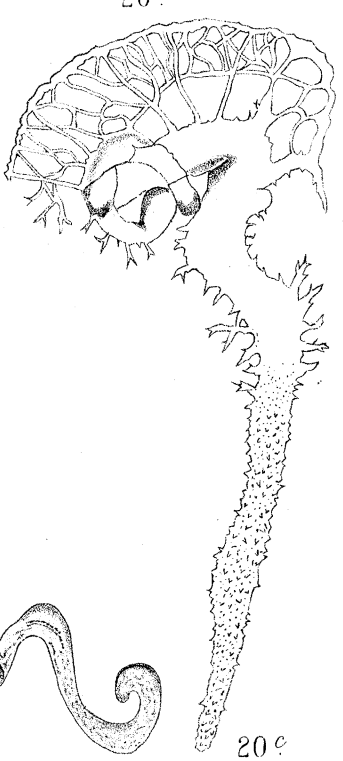
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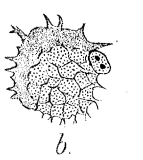
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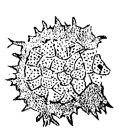
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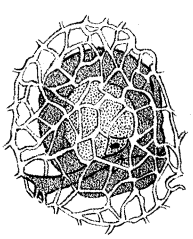
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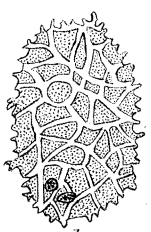
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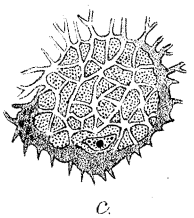
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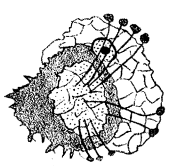
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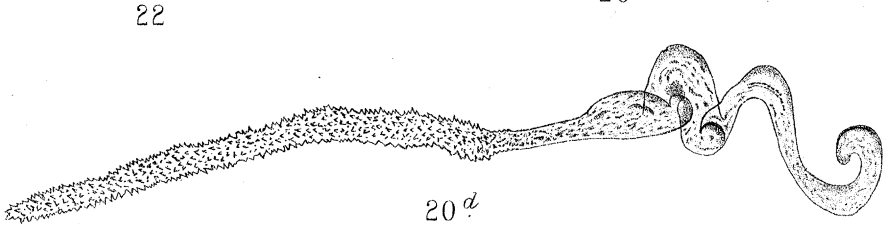
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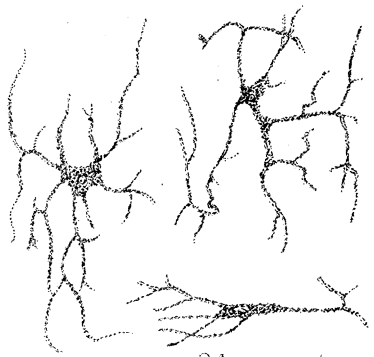

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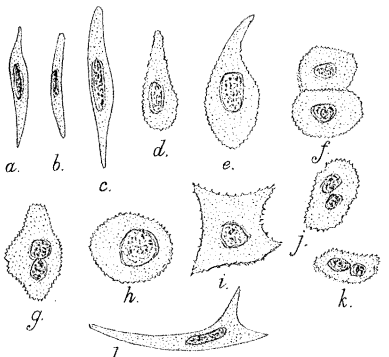

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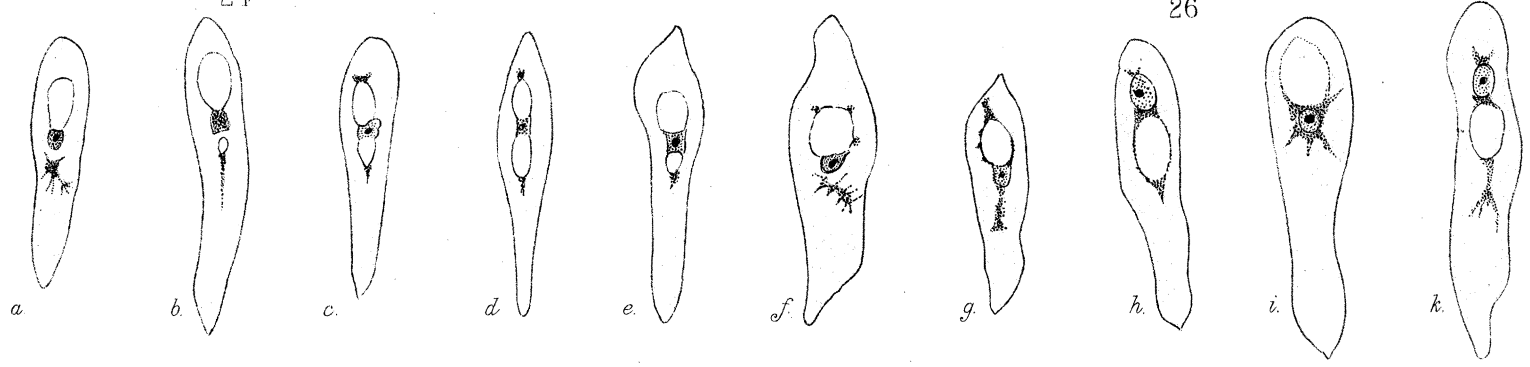

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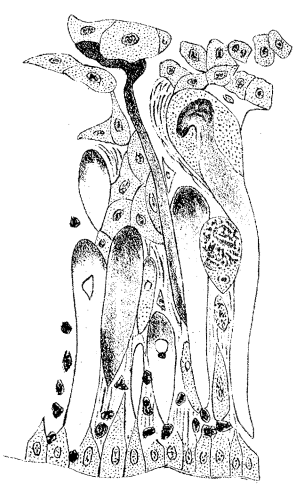

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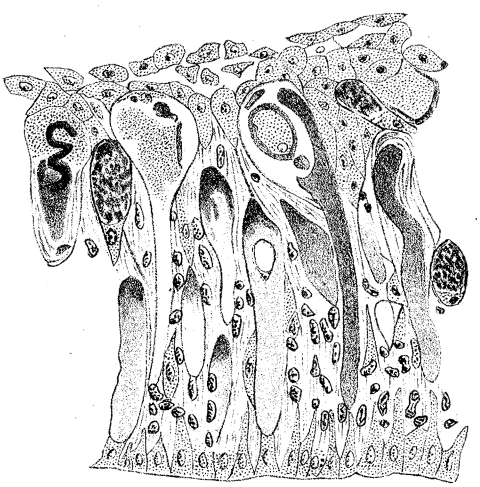

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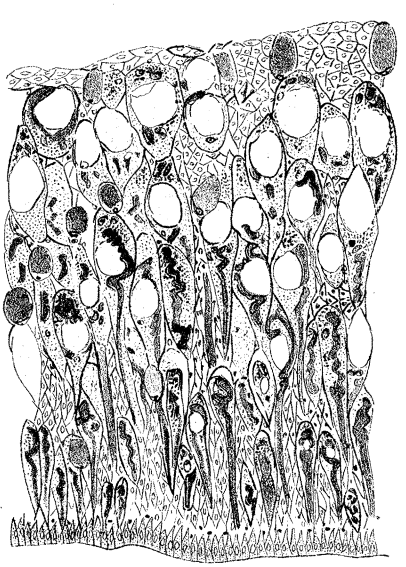

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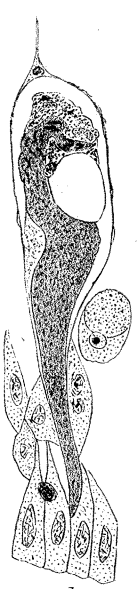

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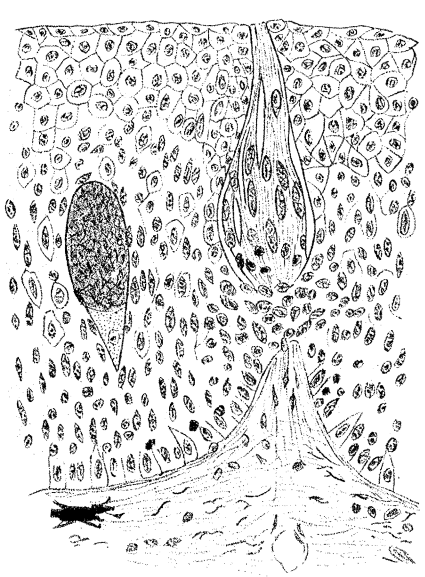

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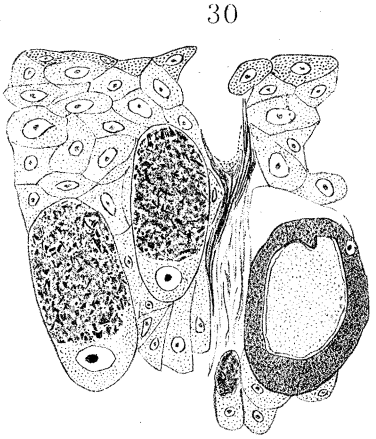

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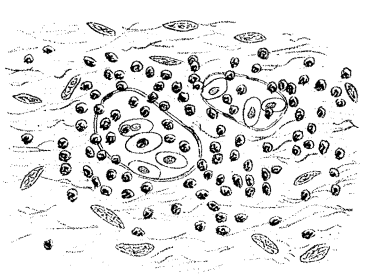

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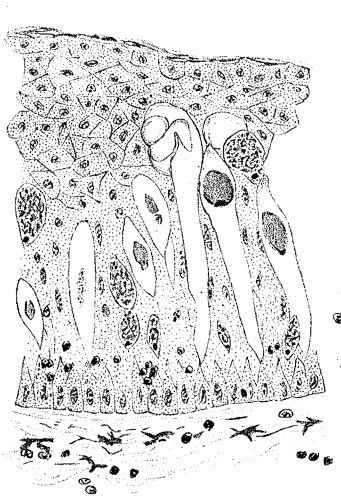

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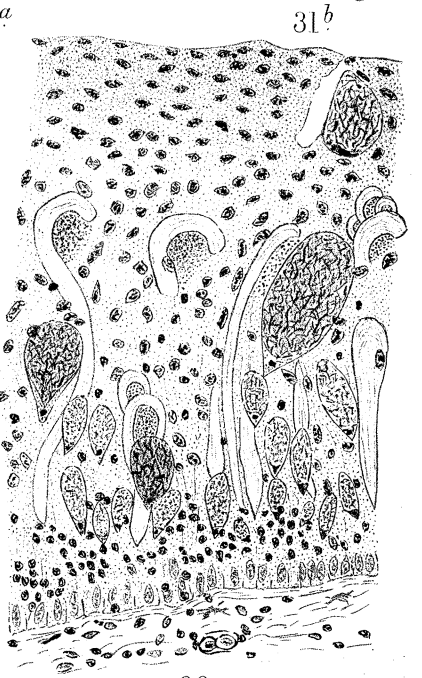

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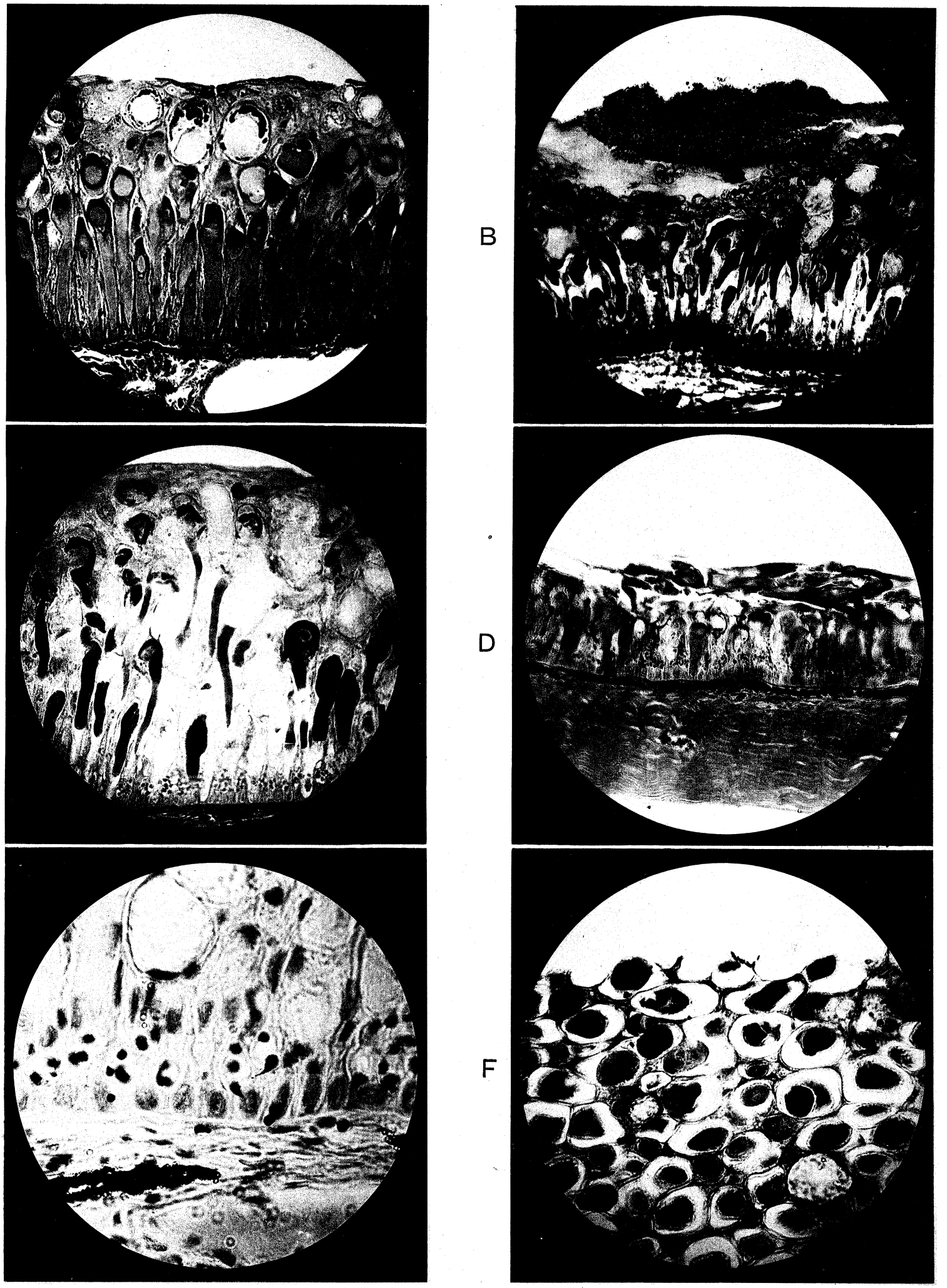
E. Waymouth Reid del^t et pinx^t.



E. Waymouth Reid.

PLATE 30.

Fig. 1. × 600. From a "cover-glass preparation" of the slime of a "normal" Eel, stained with picro-carmine. The characteristic yellow reaction of the threads of the slime is shown, also relative size of extruded nuclei and granules. An extruded goblet cell is present on the left.

Fig. 2. × 600. Origin of club cells from palisade cells. FLEMMING'S fluid and saffranine; *a*, *b*, *c*, and *f* show the method of origin by lateral division of the palisade cell at right angles to its longer axis. The darkening of the apex of the young club cell by the FLEMMING'S fluid is visible in all cases.

Fig. 3. × 600. Stage in development of club cells previous to the formation of the vesicle. Fixing and staining as in fig. 2. Note the granular matter densely stained with the saffranine, in the region of the nucleus.

Fig. 4*a* and *b*. × 600. Two stages in the development of the slime fibres from the "fibre-masses" of the club cells. From a "cover-glass" preparation of an epidermis stimulated by chloroform vapour. RANVIER'S alcohol and picro-carmine. The same yellow reaction is evident as in the formed fibres of the slime (fig. 1) in both cases, and in *b* the breaking up into the finest fibres is seen.

Fig. 5. × 600. Developed "fibre-masses" in process of extrusion. From a winter Eel that had struggled during killing. FLEMMING'S fluid and saffranine. Note the dark coloration of the fully-formed "fibre-mass," and compare with that of the apices of the club cells still possessing a vesicle. Also, the absence of saffranine staining of the contents of the club vesicle should be contrasted with the marked coloration of the contents of the goblet cells. Note also a considerable number of fibroblasts in the lower layers, many of which exhibit mitotic figures.

Fig. 6*a*, *b*, and *c*. × 600. Similarity in staining reaction of the contents of the club vesicle at early and later stages with that of the "escape mass." Winter Eel, FLEMMING'S fluid and soluble blue. The contents of the fully developed vesicle and "escape mass" are distinctly granular, that of the young vesicle is homogeneous.

Fig. 7. × 600. Division of palisade cells. FLEMMING'S fluid and saffranine. The upper examples are from sections parallel to the surface of the skin, the lower are cut at right angles to the surface. In the upper note the lateral bulging, staining yellowish-brown, and indicative of the commencement of differentiation of the club cell body material. In the lower, the deeper staining of the apices of the conical palisade cells is noticeable. No mitotic figures are to be seen.

Fig. 8. × 600. Medium stage in the development of a club cell and a goblet cell. FLEMMING'S fluid and saffranine. In the vesicle of the club cell, note a curved line, separating a denser lower part from a less dense upper part. This line passes upwards, as development proceeds, and probably marks the edge of the membrane of the "escape cell," which is becoming differentiated from the wall of the club. Note the "foot" and marked nucleolus of the goblet cell, its granular contents, and difference in staining reaction to those of the club vesicle. The dividing palisade cell immediately below the goblet cell is giving rise to an ordinary epidermic cell.

Fig. 9*a* and *b*. × 600. Origin of goblet cells from palisade cells. FLEMMING'S fluid and saffranine.

9*a*. The transformation of some of the protoplasm of the young goblet cell into a saffranine staining mucigen, even before the new cell has parted from its mother, is seen in 1, 2, and 4, and should be contrasted with the early stages and staining reactions of the young club cells in fig. 2. 2 is a case of origin by lateral division; 1 and 4, by transverse division of the palisade cell, though in the latter case the two nuclei of the palisade cell show that a lateral division will take place shortly. 3 shows how early the characteristic shape is attained in many cases.

9*b*. 6 shows that the characteristic shape may be developed even before separation from the parent, and also (as, too, in 8) the great length to which the outwardly directed process of the palisade cell may reach. In 7, a goblet cell is being thrust upwards by an ordinary epidermic cell with characteristic elongated nucleus.

Fig. 10. × 600. Regenerating goblet cell (re-formation of mucigen). FLEMMING'S fluid. Thionin, after treatment with corrosive sublimate. Winter Eel. Note the characteristic mucin reaction with thionin in the protoplasm of this cell, and contrast with the ordinary blue colour of thionin taken by the nucleus of this cell and those of the surrounding epidermic cells. A wandering pigment cell is also to be seen.

Fig. 11. × 600. Goblet cells in earlier stages of regeneration than that in fig. 10. Preparation as in case of fig. 10. The cell on the left consists of the protoplasmic "foot" alone, containing granules staining black with osmic acid. The theca, with its load of mucigen, has disappeared, but no new supply has been yet formed, as in fig. 10. The two cells on the right indicate stages in the growth of the protoplasm of the "foot," in cases where the first load of mucigen has not been completely discharged. The darkened granules are again seen here; they are never seen in the "foot" of ripe cells (*cf.* fig. 12*a*). The nucleolus is, as a rule, absent in the regenerating cells.

Fig. 12. × 600. Ripe, discharging, and regenerating (earliest stages) goblet cells. FLEMMING'S fluid. *a* is stained with methylene blue; *b*, *c*, and *d* with hæmatoxylin. In *b* and *c*, which are discharging, the stoma of the theca and the projecting "pfropf" of mucus are seen. *a* is a cell ripe for discharge, the theca still being intact. *d* is an intermediate stage in regeneration between the cells on the extreme right and left of fig. 11.

Fig. 13. × 435. Section of winter Eel killed "instantaneously," and in lowest phase of secretory activity. FLEMMING'S fluid. Thionin, after treatment with corrosive sublimate. Note the reddish-violet reaction of the contents of the goblet cells, and compare with the absence of staining of the contents of the club vesicles and the blue colour of the nuclei throughout. A "fibre mass," tending to be spiral, is seen on the left, and a point of escape, lined by a few fibrils, is seen above. A thick layer (7 or 8 cells) of superficial epidermic cells is present.

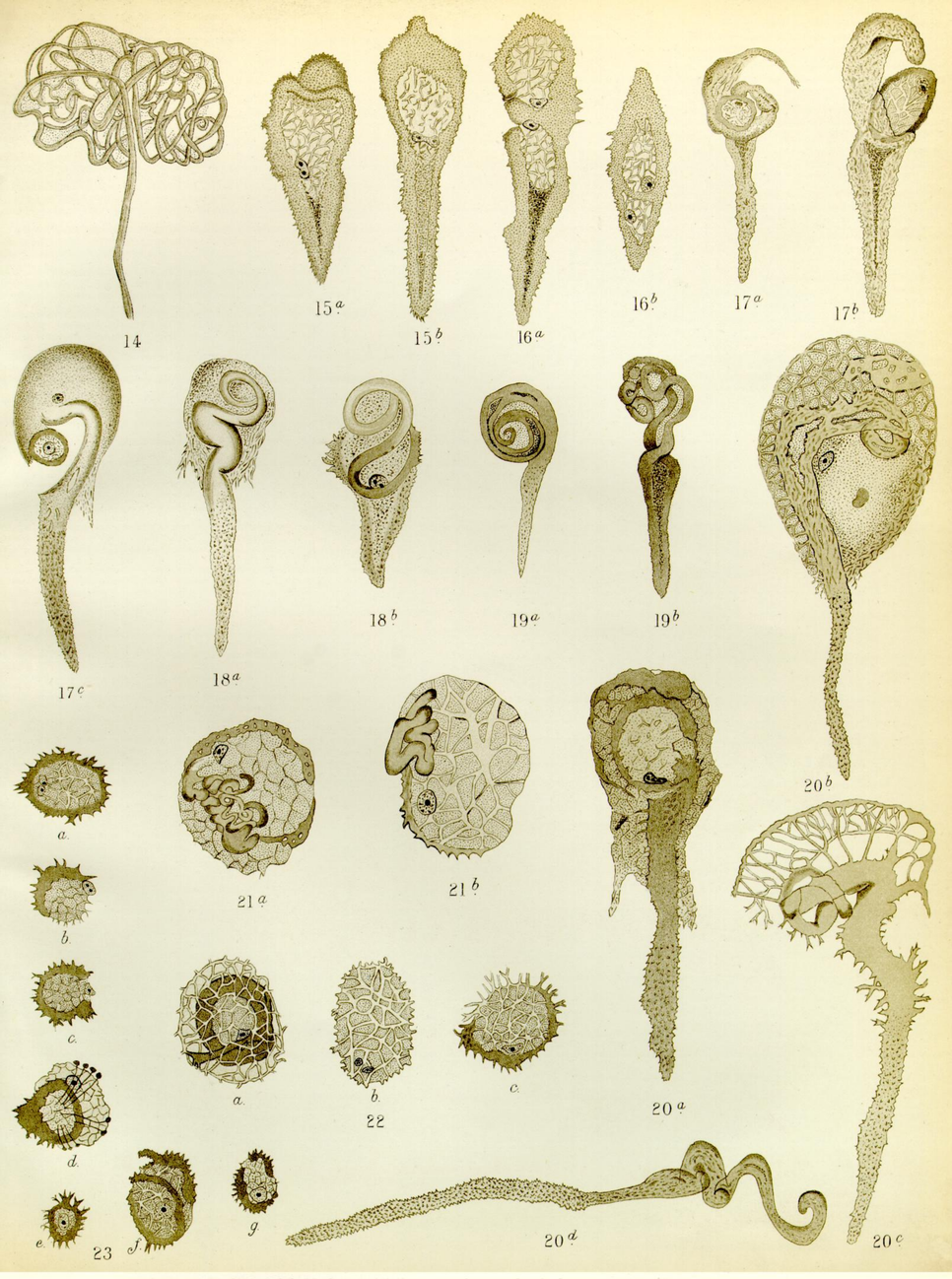


PLATE 31.

Fig. 14. × 600. Convoluted fibre, discharged by an Eel placed in a bath of pilocarpine solution. $\frac{1}{16}$-per cent. osmic. Dilute glycerine.

Fig. 15*a* and *b*. × 600. Club cells with dividing nuclei. Winter Eel. RANVIER'S "third part" alcohol. Carmine. Dilute glycerine. Note the lattice work in the wall of the vesicle and the "prickles" upon the outer surface of the body of the cell.

Fig. 16*a* and *b*. × 600. Club cells with two vesicles, each holding a nucleus. Preparation as in fig. 15. In *a*, note the granular core passing down the stalk from the vesicle.

Fig. 17*a*, *b*, and *c*. Extrusion of the "escape cell" from the club. *a* and *c*, × 435. *b*, × 600. Preparation as in fig. 15. The lattice work in the wall of the "escape cell" is especially clear in *b*. In *c*, the presence of a second nucleus indicates the possibility of formation of a second "escape cell."

Fig. 18*a* and *b*. Development of a coiled "fibre mass" without the formation of a definite "escape cell." (The remainder of the vesicle is in such cases referred to as an "escape mass" in the text.) *a*, × 435. *b*, × 600. Preparation as in fig. 15.

Fig. 19*a* and *b*. Secondary vacuolation of "fibre mass." *a*, × 435. *b*, × 600. Preparation as in fig. 15. Note granularity in secondary vacuolation spaces in *a*, similar to that occurring in the stalk in figs. 15, 16, and 17.

Fig. 20*a*, *b*, *c*, and *d*. × 600. Stages in the vacuolation of the club body material, leading up to the freeing of the "fibre mass." Preparation as in fig. 15. The vacuolation and formation of a lattice work, filled with granular matter, is seen commencing in *a*, and further developed in *b*. In *c*, this granular material has become freed in the process of maceration, so that the trellis of the residual unaltered club head material is very distinct. In *d*, the "fibre mass" is free. The "prickles" noticeable in figs. 15, 16, 17, and 18 are retained upon the lower end of the developed "fibre mass."

Fig 21*a* and *b*. × 600. Large isolated "escape cells." Preparation as in fig. 15. In this form, the greater part of the club body material is converted into granular material, and the "fibre mass" remains attached as a convoluted coarse filament.

Fig. 22*a*, *b*, and *c*. × 600. Isolated "escape cells." Preparation as in fig. 15. In *a* some of the trellis produced in the vacuolation of the club head is still adherent; *b* has no thick wall of club head material, and is an intermediate form between an "escape cell" and "escape mass." In *c* the thick wall is complete only on one side.

Fig. 23*a*, *b*, *c*, *d*, *e*, *f*, and *g*. × 600. Preparation as in fig. 15. These figures show the great variation in size and structure of "escape cells" met with in teased preparations; *a* and *b* show the possibility of division of the nucleus; *b*, *c*, and *d* show the nucleus in process of extrusion; *e* and *g* show how extremely small these cells may be in comparison with such forms as fig. 21*a* and *b*; *f* (as also in fig. 22*a*) shows a stout strand of club body material that has been ruptured in the process of "shelling out" of the cell. In all, the outer surface is seen to be beset with spines of the original lattice work—a fact also frequently clear in sections *c*, *f*, fig. 6*c*, Plate 30.

N.B.—All the figures upon this Plate, with the single exception of fig. 14, are from one winter Eel, killed "instantaneously."

PLATE 32.

Fig. 24. × 600. Pigment cells of the epidermis, from a section hardened in Flemming's fluid.

Fig. 25. × 600. Fibroblasts, isolated by Ranvier's alcohol. Carmine. Glycerine. In passing from left to right, all stages, from the simple lymphocyte-like cell to the small connective tissue cell with long fine processes, are to be observed.

Fig. 26. × 600. Ordinary epidermic cells, from various levels. Preparation as in fig. 25. *a*, *b*, *c*, and *l*, with elongated nuclei, are from the lower layers; *d* and *e*, from a higher level; *h* and *i*, from near the surface; *f*, *g*, *j*, and *k* are dividing cells from near the surface, but lack mitotic figures in their nuclei. It will be noted that the more superficial cells present "prickles."

Fig. 27. × 600. Isolated goblet cell. Preparation as in fig. 25. The plicated wall of the empty theca and the marked nucleolus of the nucleus are to be noted.

Fig. 28. × 600. Formation of the vesicle of the club cells. From sections of material hardened in Flemming's fluid, and stained with saffranine. This condition is presented in the stage following that depicted in fig. 3, Plate 30. The intimate relation of the vesicle to the nucleus is to be noted, as also the granular transformation of the club body material which precedes the enlargement of the vesicle. The vesicle may start on the outer side of the nucleus *a*, *f*, *g*, and *i*, with granularity only at the opposite pole, or *vice versâ*, *h* and *k*; on the other hand, it is possible for it to arise simultaneously upon both sides, *b*, *c*, *d*, and *e*.

Fig. 29. × 435. An escaping "fibre mass," lifting surface cells. From a winter Eel that had struggled during capture. Flemming's fluid and saffranine.

Fig. 30. × 435. An "escape cell" and related "fibre mass" *in situ*, in a section. Same Eel and preparation as fig. 29.

Fig. 31*a* and *b*. *a*, × 160. *b*, × 600. Appearances in the epidermis of an Eel that had lived two days in a solution of atropine. Flemming's fluid and saffranine. In *a*, "fibre masses" and "escape masses" are seen to exist at all levels of the epidermis, and a peculiar granularity is noticeable round about the upper ends of the "fibre masses." The general appearance of the section is suggestive of an initial secretory activity, which has been subsequently brought to a close by the paralyzing action of the drug. *b* shows the appearance of a club cell still in contact with the palisade layer, which, though developed to the full, has never left its position of origin. A developed fibroblast with long processes is seen ensheathing this club, at the upper part of the figure.

Fig. 32. × 435. Section of outer edge of lip. Picric acid and hæmatoxylin. Note the absence of club cells, presence of a goblet cell, and one of the well-known sense organs.

Fig. 33. × 600. Point of escape of a "fibre mass." From a winter Eel. Flemming's fluid and thionin.

Fig. 34. × 600. Diapedesis. From central vessels of pectoral fin. Picric acid and hæmatoxylin. These extravasated cells are quite indistinguishable from the young fibroblasts found in the lower layers of the epidermis.

Figs. 35 and 36. × 435. Fig. 35 is a section of skin subjected to the action of chloroform vapour *after removal*. Fig. 36 is from the skin of an Eel simply decapitated and then exposed to the vapour. Both were fixed with corrosive sublimate and stained with hæmatoxylin. In fig. 35, most of the club cells are in the earlier stage, with closed vesicle. In fig. 36, only curled "fibre masses" are found, with some granular débris around them. There is also to be noted, in fig. 36, a relative increase in the quantity of goblet cells and fibroblasts in the lower layers. *Cf.* also Photos B and C, Plate 33.

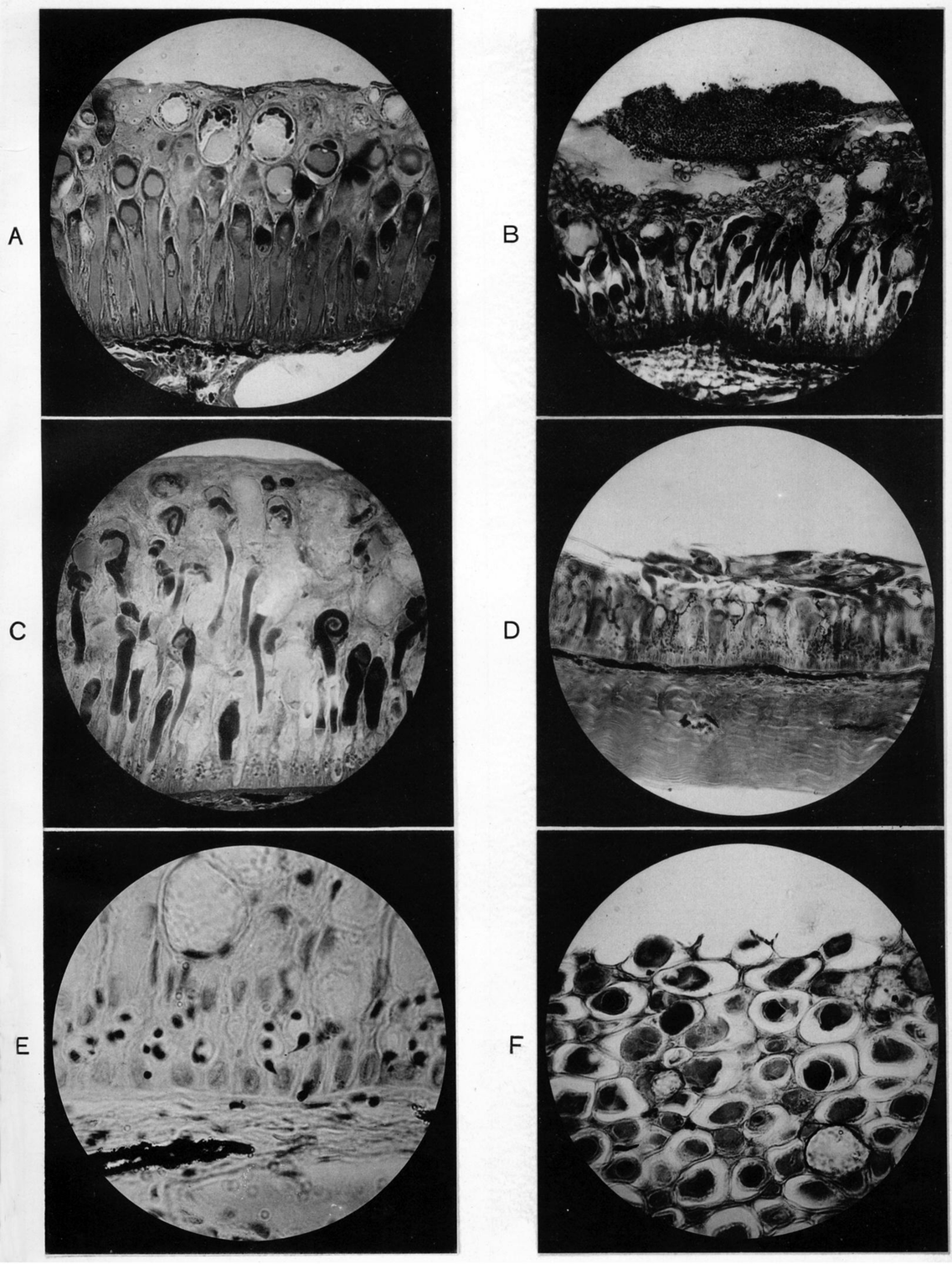


PLATE 33.

The prints on this Plate are Collotypes, direct from untouched negatives.

Photo A. × 320. Epidermis of a "normal" winter Eel. FLEMMING'S fluid and saffranine. Note below the club cells, with closed vesicles, and the conical palisade cells; above, the free "escape masses." This print represents the appearances of the lowest phase of secretory action.

Photo B. × 175. Later stages of stimulation by chloroform vapour. Nitric and osmic acid. Saffranine. Note the "eruption" of fibre masses (darkly stained) causing considerable disruption of the layers of the epidermis. Above is seen a compact mass of small cells (5μ), which are, probably, extruded fibroblasts. A pad of coagulated mucus, containing a few epidermic cells, separates this mass of small cells from the parts below. This print should be contrasted with the appearance of the resting skin seen in Photo A.

Photo C. × 300. Early stages of stimulation by chloroform vapour. Compare with Photo B. Nitric and osmic acids. Saffranine. A good example of a coiled fibre mass is seen on the right.

Photo D. × 175. Epidermis of a summer Eel faradised for an hour. FLEMMING'S fluid and saffranine. The superficial epidermic cells are absent, and the surface consists of a mass of extruded fibre masses and club cells. An escaping club cell is evident upon the left. Note the large number of fibroblasts in the lower layers.

Photo E. × 900. Passage of fibroblasts from the corium into the epidermis. Picric acid and hæmatoxylin. Fibroblasts can be seen upon the corium side of the basement membrane, one is fixed as it passes through, another between two palisade cells, and others lie free in the epidermis. In two of these latter a protoplasmic process is evident upon one side of the cell. Note the relative sizes of the nuclei of the palisade cells and the fibroblasts.

Photo F. × 450. Connective tissue network in the epidermis. Section parallel to the surface of the skin about midway between surface and basement membrane. Picric acid and saffranine. The bodies of the club cells have shrunk in preparation, and so allow the branching cells to be easily visible.